

PSYCHOSOCIAL AND BEHAVIORAL DETERMINANTS OF IMMUNE AGING

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ABSTRACT

This thesis explored the hypothesis that cytomegalovirus (CMV) infection and its reactivation may be a shared mechanism linking psychosocial and behavioral factors with the age-associated decline in immunity, known as immunosenescence. The first empirical chapter (Chapter 3) showed that psychological stress factors were positively associated with CMV reactivation, as measured by increased CMV-specific IgG antibodies (CMV-IgG) among those infected, while socioeconomic and lifestyle factors were associated with CMV infection rates. Chapter 4 investigated personality traits and revealed that increased neuroticism predicted elevated odds of CMV infection and higher conscientiousness was associated with lower CMV-IgG levels. Chapter 5 demonstrated that more frequent physical activity was associated with lower levels of highly-differentiated T-cells, but this association was reduced to non-significance by adjustment for CMV infection. Chapter 6 showed that dysregulated glucose metabolism, measured as higher glycated hemoglobin levels, was associated with increased highly-differentiated T-cells in CMV-infected individuals. Furthermore, hyperglycemia interacted with CMV infection for a further increased accumulation of these cells. In sum, these results suggest that CMV and psychosocial and behavioral factors co-determine the progression of immunosenescence, and that CMV reactivation may reflect imbalance among these factors. Thus, CMV reactivation is proposed as a common pathway in psychobiological relationships with immunosenescence.

For my family

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LIST OF PAPERS

The general introduction of this thesis is an adapted version of the following book chapter:

- Bosch, J. A., **Rector, J. L.**, Turner, J. E., Riddell, N. E., ó Hartaigh, B., & Burns, V. E. (2012). Psychoneuromicrobiology: Cytomegalovirus Infection as a Putative Link between Stress, Aging, and Immunity. In J. A. Bosch, A. C. Phillips & J. M. Lord (Eds.), *Immunosenescence: Psychosocial and Behavioral Determinants* (pp. 81-100). New York: Springer.

This thesis incorporates the following four papers:

1. **Rector, J. L.**, Dowd, J. B., Loerbroks, A., Burns, V. E., Moss, P. A., Jarczok, M. N., Stalder T., Hoffman K., Fischer J. E., Bosch, J. A. (2014). Consistent associations between measures of psychological stress and CMV antibody levels in a large occupational sample. *Brain Behav Immun*. doi: 10.1016/j.bbi.2014.01.012
2. **Rector, J. L.**, Burns, V. E., Moss, P. A., Jarczok, M. N., Hoffman K., Fischer J. E., Bosch, J. A. (in preparation). Personality and cytomegalovirus.
3. **Rector, J. L.**, Simpson, R. J., Burns, V. E., Moss, P. A., Jarczok, M. N., Hoffman K., Fischer J. E., Bosch, J. A. (in preparation). Association between physical activity frequency and highly-differentiated T-cells.
4. **Rector, J. L.**, Burns, V. E., Moss, P. A., Jarczok, M. N., Hoffman K., Fischer J. E., Bosch, J. A. (in preparation). Elevated HbA1c and the accumulation of highly-differentiated T-cells in CMV+ individuals.

In addition the following presentations arose from material from this thesis:

- Neuroendocrine Immune Networks in Ageing (NINA) 1st, 2nd, & 3rd Annual Meeting. Munich, Germany, October 2011; Paris, France, November 2012; Amsterdam, The Netherlands, June 2013. *Stress, immunity, and mental health II – Stress in adulthood*
- American Psychosomatic Society 71st Annual Scientific Meeting. Miami, FL, USA, March 2013. *Consistent Associations between Measures of Distress and CMV Reactivation in a Large Occupational Sample*
- 3rd International Workshop on CMV & Immunosenescence. Córdoba, Spain, March 2012 *Clustering of Socioeconomic, Lifestyle, and Psychological Risk Factors in CMV+ Individuals*

Lastly, work included in this thesis received the following media coverage:

- *How to live longer – the experts' guide to ageing*. Interview in The Guardian by Catherine de Lange, Sunday 8 September 2013.

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ABBREVIATIONS

β 2AR – β ₂-adrenergic receptor
 $\gamma\delta$ TCR – gamma delta T-cell receptor
AIDS – acquired immune deficiency syndrome
ANCOVA – analysis of covariance
APC – allophycocyanin
APC-Cy7 – allophycocyanin-cyanine dye 7
AUC – area under the curve
BMI – body mass index
BP – blood pressure
CAR – cortisol awakening response
CD – cluster of differentiation
CI – confidence interval
CM – central memory
CMV – cytomegalovirus
CMV– – CMV-negative
CMV+ – CMV-positive
CMV-IgG – CMV-specific IgG antibody
CTL – cytotoxic T lymphocyte
CVD – cardiovascular disease
dCTL – differentiated cytotoxic T lymphocytes
dept – department
DHEA – dehydroepiandrosterone
DNA – deoxyribonucleic acid
EBV – Epstein-Barr virus
EDTA – ethylenediaminetetraacetic acid
ELISA – enzyme-linked immunosorbent assay
EM – effector memory
EMRA – CD45RA revertant effector memory
FACS – fluorescence-activated cell sorting
FCS – flow cytometry standard format
FITC – fluorescein isothiocyanate
GLUT – glucose transporter
HADS – Hospital Anxiety and Depression Scale
HbA1c – glycated hemoglobin
HDL-C – high density lipoprotein cholesterol
HIV – human immunodeficiency virus
HPA – hypothalamic-pituitary-adrenal
HR – heart rate
hs-CRP – high-sensitivity c-reactive protein
HSV-1 – Herpes simplex virus 1
HSV-2 – Herpes simplex virus 2
ICU – intensive care unit
IE – immediate-early
IFN- γ – interferon gamma
IgA – immunoglobulin A

IgG – immunoglobulin G
IgM – immunoglobulin M
IL – interleukin
kcal – kilocalories
LDL-C – low density lipoprotein cholesterol
mgr – manager
MICS – MIPH Industrial Cohort Studies
MIPH – Mannheim Institute of Public Health
MONICA – MONItoring of trends and determinants in CArdiovascular disease
MQ – Maastricht Exhaustion Questionnaire
NA – naïve
NEO-PI-R – NEO Personality Inventory-Revised
NK – natural killer
OR – odds ratio
PA – physical activity
PE – phycoerythrin
PE-Cy7 – phycoerythrin-cyanine dye 7
PerCP – peridinin chlorophyll protein
PTSD – post-traumatic stress disorder
qPCR – quantitative polymerase chain reaction
SEM – standard error of the mean
SAM – sympathetic-adrenal-medullary
SD – standard deviation
SE – standard error
SES – socioeconomic status
SF-12 – 12-item Health Survey – short form
TCR – T-cell receptor
TNF- α – tumor necrosis factor alpha
URI – upper respiratory infection
US – United States of America
UV – ultraviolet
VE – vital exhaustion
VZV – Varicella Zoster virus

CHAPTER 1 : GENERAL INTRODUCTION

Introduction

Epidemiological evidence demonstrates increased morbidity and mortality in populations exposed to adverse psychosocial factors such as low socioeconomic status and protracted psychological distress (Cohen & Herbert, 1996; House et al., 1988; Marmot, 2005; Schneiderman et al., 2005). While the data is clear, the precise mechanisms underlying these associations have yet to be determined (Antoni et al., 2006; Cacioppo & Hawkley, 2003; Glaser & Kiecolt-Glaser, 2005; McEwen, 1998; Uchino et al., 1996). It has been argued that since increasing age is a major risk factor for a wide range of chronic diseases, the aging process itself may be an important target for such mechanistic research (Bauer et al., 2013; Bosch et al., 2009; Nilsson, 1996).

Immunosenescence

This thesis explores the human herpes virus, cytomegalovirus (CMV), as a possible biological pathway linking psychosocial stress and immunosenescence, which may underlie psychobiological associations with health (Bosch et al., 2009).

Immunosenescence refers to a decline in immune competence seen in old age, and it is associated with a dramatic rise in morbidity and mortality from infectious disease (Akbar et al., 2004; Larbi et al., 2008). For example, the elderly exhibit a many-fold higher mortality from otherwise common infections such as gastrointestinal infections, urinary tract infections and influenza (Thompson et al., 2003; Yoshikawa, 2000). This susceptibility also promotes physical frailty and cognitive decline, further increasing mortality risk and reducing quality of life (High et al., 2005; Yoshikawa, 2000). Although less well established, immunosenescence may also be a factor in the age-related increase in

autoimmune and inflammatory disorders (Franceschi, Bonafe, et al., 2000; Hohensinner et al., 2011; Lindstrom & Robinson, 2010; Prelog, 2006).

These clinical complications of immunosenescence mark the terminal phase of a process that already starts in adolescence (Akbar & Fletcher, 2005; Nikolich-Zugich, 2008). Around that time the thymus, an organ essential for the maturation of T lymphocytes, reduces in size and function with a concomitant decline in the generation of naïve T-cells, partially due to increased circulating sex hormones during puberty (Appay et al., 2010; Palmer, 2013). This process causes immunity to become progressively dependent on the existing pool of naïve and memory T-cells (Woodland & Blackman, 2006). The pool of memory T-cells gradually expands, compensating for the diminishing influx of naïve T-cells via cytokine-induced cell division (denoted ‘homeostatic proliferation’) and repeated antigen exposure (Pawelec, 2012). In particular latent herpes viruses, like CMV, are a major source of continuous antigenic stimulation (Khan et al., 2002; Nikolich-Zugich, 2008). Consequently, the expanding pool of memory cells tends to be oligoclonal, i.e., exhibit an antigen receptor diversity that is skewed towards a few immunodominant viral antigens. These changes are particularly prominent in CD8⁺ T lymphocytes (CTLs) and the accumulation of these oligoclonal T-cells is considered a hallmark of immunosenescence (Akbar & Fletcher, 2005; Hadrup et al., 2006; Pawelec, 2006).

T-cell phenotypes, their development, and functional characteristics

The general observation is that repeated contact with the same antigen moves memory cells along a differentiation continuum whereby they gradually change their cell-surface appearance, or phenotype, and acquire so-called ‘effector’ functions, such as increased cytotoxicity (as determined by granzyme and perforin expression), and attain a

preference to home to peripheral tissue (e.g., the skin and lungs) rather than the lymphoid tissue. Immunologists utilize various analytical strategies based on the staining of cell-surface molecules to identify CD8⁺ T-cell ‘subsets’ at distinct stages of this differentiation process. The combination of the markers CD45RA (an isoform of the pan-lymphocyte marker CD45) and the co-stimulatory molecule, CD27 (gradually lost with increased differentiation), has been chosen for this thesis. This approach can be used to discern antigen inexperienced, or naive, cells (NA; CD27⁺CD45RA⁺), and three additional subsets of antigen-experienced (‘memory’) cells: central memory (CM; CD27⁺CD45RA⁻), effector memory (EM; CD27⁻CD45RA⁻) and effector memory cells which have re-expressed CD45RA (EMRA; CD27⁻CD45RA⁺) (Appay et al., 2008; Hamann et al., 1997; Sallusto et al., 2004; Sallusto et al., 1999; Van Lier et al., 2003). This method of subset identification was chosen because the EMRA T-cell phenotype is associated almost exclusively with CMV infection, and may reflect recent CMV activity (Cantisán et al., 2010; Chidrawar et al., 2009; Derhovanessian et al., 2011; Kuijpers et al., 2003; Turner et al., 2014; Van De Berg et al., 2008; Van Lier et al., 2003). The EM and EMRA subsets have undergone several rounds of cell division and are considered to be highly-differentiated T-cells.

Highly-differentiated T-cells contribute to features of immunosenescence

Markers of an aging adaptive immune system include weakened cell-mediated and humoral responses to vaccination, a diminished proliferative response to mitogens, a reduced production of interleukin-2 (IL-2; which stimulates the growth, differentiation and survival of cytotoxic T-cells), shortening of leukocyte telomeres (a DNA marker of cellular aging), increased serum levels of inflammatory cytokines like tumor necrosis factor alpha (TNF- α), persistent reactivation of latent herpes viruses, e.g., Epstein-Barr Virus (EBV) and CMV, and a reduced CD4:CD8 ratio (Effros, 2007; Goronzy & Weyand,

2013; Larbi et al., 2008; Luz Correa et al., 2014; Pawelec, 2006; Strindhall et al., 2012; Turner et al., 2014). Significantly, all of the aforementioned immune changes that characterize aging are, in part, a result of the accumulation of T-cells with a differentiated phenotype (i.e., EM and EMRA); that is, these T-cells poorly proliferate in response to mitogens, produce little IL-2, but abundant inflammatory interferon gamma (IFN- γ), have very short telomeres, expand in response to repeated latent viral reactivation and the number of these cells correlate with weaker vaccination responses (Monteiro et al., 1996; Nikolic-Zugich, 2008; Van De Berg et al., 2010).

Stress effects parallel those of immune aging

Those who are familiar with the psychoneuroimmunology literature may immediately note a striking similarity between the immune changes summarized in the previous paragraph and the immune effects of chronic stress (Bosch et al., 2009). Indeed, meta-analysis has shown that protracted psychosocial stressors are associated with much the same pattern of weakened responses to immunization, diminished proliferation to mitogens, reduced IL-2 production, and reactivation of latent herpes viruses (Segerstrom & Miller, 2004). Other distinctive features of immunosenescence, such as elevated serum levels of inflammatory markers and shortened leukocyte telomeres, have also consistently been associated with psychosocial stress (Damjanovic et al., 2007; Epel et al., 2004; Glaser & Kiecolt-Glaser, 2005; Simon et al., 2006). The remarkable parallels between the aging and stress literature leads one to hypothesize that psychosocial stress contributes to the process of immunological aging (Bosch et al., 2009). Thus, the hypothesis explored in the current thesis is that the effects of stress and aging on immunity not only just look the same, but may also share similar pathways. Moreover, it will be argued that one of these shared pathways may be the effects of cytomegalovirus infection on immunity. The sections that follow will describe how CMV infection dramatically alters the composition

of the blood, causing an accumulation of highly-differentiated CD8⁺ T-cells (CD27⁻); a hallmark of an aging immune system.

Cytomegalovirus infection and immunity

It is thought that this CMV-driven accumulation of differentiated cells is due to repeated low-grade viral reactivation. Viral reactivation activates CMV-specific T-cells, which as a population will both expand (i.e., forming a gradually larger portion of the total pool of memory T-cells) and differentiate (i.e., acquire potent cytotoxic capabilities). Significantly, there is evidence that psychological and psychosocial stressors, via neuroendocrine and immunological pathways, may contribute to this viral reactivation, implying that stress exposure may promote this aspect of immunosenescence.

Herpes infections and latency

Viruses are microscopic pathogens, consisting of a nucleic acid genome inside a protein coat, which can only replicate once inside a living cell. While the immune system is able to eradicate most viral infections, some viruses have developed ways to escape this fate. The herpes viruses are particularly well-known for this ability to evade immune destruction and persist within their human host (Cohrs & Gilden, 2001; Croen, 1991; Froberg, 2004; Miller-Kittrell & Sparer, 2009). A characteristic of herpes viruses is that they remain dormant in the host, a state denoted as latency, which is interrupted by brief periods of reactivation whereby the virus replicates and infects other cells. Herpes viruses have been extremely successful in colonizing humans. Their typical infection rates range between 30% and 90% of developed countries, depending on the virus, and are, in part, related to factors such as age, socioeconomic status, geographical location, sexual experience, and early life exposures (e.g., higher infection rates in children that attended day care) (Arvin et al., 2007). These viruses employ numerous evasion tactics, such as

producing anti-inflammatory IL-10 homologues, in order to avoid immune detection and increase survival that can affect immunity (Froberg, 2004). In healthy individual these viruses typically elicit mild (e.g., cold sores, fatigue) or no disease symptoms at all. However, in immune-suppressed patients (e.g., those with AIDS or using immune suppressive drugs), these viruses can elicit very severe complications (Nester et al., 2008). Eight herpes viruses have thus far been identified – herpes simplex virus 1 (HSV-1), herpes simplex 2 (HSV-2), varizella zoster virus (VZV), Epstein-Barr virus (EBV), Roseola virus, T-cell lymphotropic virus, Kaposi's sarcoma-associated herpes virus, and finally, CMV, which is the focus of this thesis.

CMV: epidemiology and pathology

CMV is a highly prevalent herpes virus; approximately 35% of young children (6-11 years) and 90% of the elderly (≥ 80 years) are seropositive (Staras et al., 2006). Overall sero-prevalence in western societies approaches 60% (Staras et al., 2006). Individuals free from infection at birth will likely become infected early in life: post-natal or childhood CMV infection is common, and caused by exchange of bodily fluids, including breast-milk (Kenneson & Cannon, 2007; Van Der Meer et al., 1996). An inverse relationship exists between socioeconomic status and CMV seropositivity. Though the exact mechanisms are unknown, individuals with less education, lower income, and of non-white race are more likely to become infected, and at an earlier age, than individuals of a higher socioeconomic status (Dowd & Aiello, 2009; Dowd et al., 2009). Studies have also reported differences in human CMV infection between various regions within the US and Europe (Bate et al., 2010; Cannon et al., 2010; Staras et al., 2006).

As a common, asymptomatic and seemingly innocent infection, CMV has consequently not received much public health attention (Wregghitt et al., 2003; Zanghellini

et al., 1999). However, this insidious virus may be more damaging than previously thought. Cytomegalovirus infection during pregnancy, for example, is now recognized as a main cause of infant death and long-term disabilities in the US, and well exceeds that of other, better known, congenital conditions such as Down syndrome, fetal alcohol syndrome, or spina bifida (Cannon & Davis, 2005; Cheeran et al., 2009). Further, immune-compromised individuals, such as HIV patients and transplant recipients, can develop life-threatening complications due to CMV reactivation (Freeman, 2009; Sutherland et al., 1992). In aging populations, a positive CMV serotatus has been associated with cognitive decline, and is implicated in the pathogenesis and severity of cardiovascular diseases (Aiello et al., 2006; Söderberg-Nauclér, 2006). In addition, the extent of CMV infection, measured by the concentration of immunoglobulin G (IgG) antibodies to CMV, independently predicts mortality in older adults (Roberts et al., 2010; Strandberg et al., 2009). Thus, this once assumed inconsequential virus has a range of deleterious effects, particularly within the immune system. The next section will outline these effects, which are particularly prominent in CD8⁺ T lymphocytes.

The effects of CMV infection on immunity

As discussed above, one of the hallmarks of an aged immune system is a decline in naïve (CD27⁺CD45RA⁺) CD8⁺ T-cells and an accumulation of differentiated (CD27⁻CD45RA^{+/-}) CD8⁺ T-cells. These effects are partly caused by the age-associated involution of the thymus. For example, individuals thymectomized in the first few years of life exhibit reduced numbers of naïve T lymphocytes and increased numbers of late-differentiated cells (Eysteinsdottir et al., 2004; Sauce et al., 2009; Torfadottir et al., 2006). However, it is now clear that CMV is the major driving force behind the accumulation of differentiated T-cells (Moss, 2010; Pawelec et al., 2004; Pawelec & Derhovanessian, 2010; Pawelec et al., 2009). In particular, the combination of little or no thymic output, a

hallmark of aging, and a selective expansion of highly-differentiated, CMV-specific T-cell populations leads to a gradual overcrowding by CD8⁺ T-cells that have a limited T-cell repertoire, a process denoted as memory inflation (Akbar & Fletcher, 2005; Brunner et al., 2010; Sauce et al., 2009; Van Lier et al., 2003). Remarkably, in some older adults it has been observed that up to 70% of the CD8⁺ T-cell memory pool has become specific for CMV epitopes (Appay et al., 2002; Khan et al., 2002), and thus only 30% of the memory pool of those individuals is available to combat other antigens. The relation between CMV infection and CD8⁺ T-cell differentiation occurs independent of calendar age: young adults infected with CMV similarly exhibit skewing of the T-cell repertoire as seen in older adults (Chidrawar et al., 2009; Pita-Lopez et al., 2009; Turner et al., 2014; Weinberger et al., 2007). Thus, it seems that infection with this common micro-organism accelerates the immunological aging process.

As noted above, these highly-differentiated CD8⁺ T-cells are also efficient producers of inflammatory cytokines (e.g., TNF- α and IFN- γ ,) (Clerici et al., 2001; Macaulay et al., 2013; Sansoni et al., 2008; Zanni et al., 2003), which may explain why CMV infection and the accumulation of late-differentiated T-cells has been found to be associated with increased low-grade inflammation in some studies (Markovic-Plese et al., 2001; Schmidt et al., 1996; Söderberg-Nauclér, 2006; Sun et al., 2008; Wikby et al., 2006; Zanni et al., 2003).

Together, these effects of CMV on the accumulation of T-cells with the above cluster of characteristics may underlie heightened inflammation, increased risk for infection and reduced ability to respond to novel antigens, as well as accelerated cognitive decline (Larbi et al., 2009; Saurwein-Teissl et al., 2002; Trzonkowski et al., 2009; Wikby et al., 2005). It is perhaps relevant to reiterate here that this cluster of immunological

features is also characteristically found in response to protracted psychological stress (Segerstrom & Miller, 2004).

Measuring CMV reactivation

Most studies that investigated biological markers of CMV reactivation used plasma levels of virus-specific IgG as an outcome. This measurement approach is based on the assumption that an increased viral load will activate the immune system, thereby stimulating B-cells to increase the output of specific antibodies. The validity of the studies using antibody titers as an outcome measure appears corroborated by research that used other analytical techniques. For example, Kuo et al. (2008) measured CMV viral load using real-time quantitative polymerase chain reaction (qPCR) and CMV-specific IgG antibody levels among patients undergoing immunosuppressive chemotherapy treatment. In most patients, the peak in CMV antibodies was tightly coupled with peak viral loads, indicating an active response to CMV replication (Kuo et al., 2008). While there is support for the validity of this assumption, mainly from clinical data, it may be relevant to point out that other factors may also determine antibody levels. For example, exposure to different variants of the same virus, denoted as ‘super-infection’, may generate a more extensive polyclonal antibody response and concomitantly higher virus-specific antibody levels.

The number of late-differentiated T-cells is related to viral activity

One key assumption of the model presented here is that repeated viral reactivation, e.g., as a result of stress-induced immune suppression, will promote the accumulation of late-differentiated cells and thereby promote the related features of immunosenescence. Virus-specific T-cells, such as those targeting CMV, are responsible for preventing reactivation and it would therefore be reasonable to assume a direct correlation between

the number of those cells and viral load (Ogg et al., 1998; Seckert et al., 2012; Van Baarle et al., 2002). Research shows that immune-suppressed individuals (e.g., as a result of medical treatment or immunodeficiency diseases) are unable to maintain CMV in latency, and show larger expansions of late-differentiated T-cells relative to compared to immune-competent individuals (Gamadia et al., 2001). Less data is available for healthy free-living individuals, and recipients of organ transplants have been frequently used as a model to study the kinetic effects of infection and reactivation on T-cell repertoire. CMV-seronegative patients receiving CMV-infected renal transplants develop primary CMV infections, and can therefore be studied longitudinally to examine the effects of CMV infection and incessant viral reactivation. Generally, an increase in viral activity (as assessed by viral load in plasma, or IgG antibodies to CMV) promotes a compensatory increase in the number of CMV-specific T-cells which do not express CD27 or CD28, including gamma-delta ($\gamma\delta$) T-cells (Cantisán et al., 2009; Couzi et al., 2009; Gamadia et al., 2003; Gamadia et al., 2004). As mentioned above, the selective accumulation of EMRA T-cells appears unique to CMV infection, providing a strong empirical basis to suggest that these cell populations are reflections of CMV activity (Cantisán et al., 2010; Chidrawar et al., 2009; Derhovanessian et al., 2011; Kuijpers et al., 2003; Turner et al., 2014; Van De Berg et al., 2008; Van Lier et al., 2003). Thus, frequent reactivation of CMV, possibly by psychological stress (Coskun et al., 2010; Prosch et al., 2000; Sarid et al., 2001), might further exacerbate immunosenescence. The section below will highlight the possible mechanisms allowing for CMV reactivation.

Potential mechanisms of stress-induced CMV reactivation

The dominant hypothesis in psychoneuroimmunology is that latent viral reactivation is due to temporary or persistent stress-induced immune suppression. This immune suppression, in turn, may be related to dysregulation (typically hyperactivity) of

various neuroendocrine stress systems (Bauer, 2005; Bauer et al., 2000). Best studied in this regard are the hypothalamic-pituitary-adrenal cortex (HPA) system, which regulates the release of glucocorticoids, and the sympathetic-adrenal-medullary (SAM) nervous system, which regulates the release of the catecholamines adrenaline and noradrenaline (Glaser & Kiecolt-Glaser, 2005). While there is little doubt that this prototypical psycho-neuro-immunological pathway plays a role in viral reactivation, more direct pathways may be involved as well.

One example, discussed in more detail here, is direct activation of the CMV promoter region by mediators of the stress response. The CMV promoter is a gene segment that contains binding sites for transcription factors that are increased during inflammation, β -adrenergic stimulation and reactive oxygen species production, and regulates the expression of downstream major immediate-early (IE) genes. Expression of these genes plays an important role in the initial steps leading to reactivation of CMV from latency (Hermiston et al., 1987; Stenberg et al., 1984; Stinski & Isomura, 2008). Thus, stress-related elevations in these factors may directly trigger CMV to reactivate. Systemic inflammation, particularly TNF- α , has been associated with CMV reactivation in various patient groups, including cardiovascular, transplant patients (Humar et al., 1999; Tong et al., 2001; Widmann et al., 2008) and intensive care unit (ICU) patients (Chilet et al., 2010; Limaye & Boeckh, 2010; Limaye et al., 2008). Experimental evidence demonstrates that elevated sympathetic activation, and the concomitant release of the catecholamines, adrenaline and noradrenaline, are likewise capable of inducing CMV reactivation in these cells, as measured by expression of the IE gene (Docke et al., 1994; Montminy, 1997; Prosch et al., 1999; Prosch et al., 2000; Stein, Volk, Liebenthal, Kruger, et al., 1993). In addition, stress-induced elevations in blood glucose and other metabolic factors can result in the production of reactive oxygen species (Cohen et al., 2011; Epel, 2009), which have

been shown to cause an up to 3-fold increase in promoter activation *in vitro* (Jaganjac et al., 2010). These findings represent important non-immunological pathways by which stress may cause CMV reaction. It should be noted that although expression of the IE genes are a first and essential step in reactivation, it is not sufficient for full viral replication, and therefore the definitive evidence for this mechanism still needs to be established.

Summary of the current literature

While much research effort has been dedicated to identifying the biological determinants of immunosenescence, the potential role of psychosocial and behavioral factors remains ill-considered. This neglect seems unwarranted considering the remarkable similarities between the functional and phenotypical immune changes observed during immunosenescence and those seen in response to protracted psychosocial stress. These changes include increases in circulating EM and EMRA CTLs, impaired proliferation in response to mitogens, shortening of telomeres, diminished response to vaccination, and a skewed CD4:CD8 ratio. These parallels beg the question of whether the overlapping age- and stress-related immune effects involve similar pathways. The next section will address the evidence for how specific psychosocial and behavioral factors may activate these mechanisms and thereby promote CMV reactivation.

Psychosocial and behavioral factors and CMV reactivation

There is compelling evidence that links between psychosocial and behavioral factors and immunosenescence may share CMV as a common pathway. Particularly, psychological stress, personality, physical activity and glucose metabolism have received much research attention, and the results show remarkably consistent associations between these factors, health and longevity (Barr et al., 2009; Chapman et al., 2011; Woodcock et

al., 2011). Each of these factors may also enhance or reduce CMV infection and/or its reactivation. It is this interaction with CMV activity that may tie these seemingly unrelated factors together and underlie their individual associations with the acceleration, delay or possible mitigation of various aspects of immunosenescence (see Figure 1.1). Below, I will briefly introduce the factors explored in this thesis and describe the current evidence linking each to CMV infection and/or reactivation.

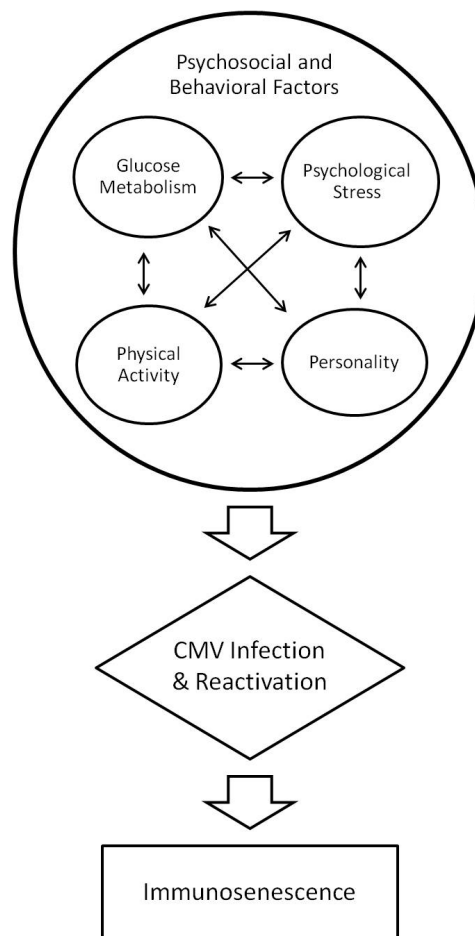


Figure 1.1: Psychosocial and behavioral factors share CMV and its reactivation as a pathway to immunosenescence

Psychological stress

The stress literature has utilized a wide variety of stressor models, such as elevated anxiety and depressive symptoms (Phillips et al., 2008), academic exams (Bosch et al., 2001; Bosch et al., 2003), Alzheimer caregiving (Glaser & Kiecolt-Glaser, 2005), space flight (Mehta et al., 2000), and post-traumatic distress disorder (PTSD) (Gill et al., 2009). Table 1.1 and 1.2 below summarize findings from studies associating these various measures of psychological stress and CMV reactivation. The results of these studies paint a fairly consistent picture whereby higher levels of distress are associated with more CMV reactivation. This association is in line with what has been found in studies on other herpes viruses such as Epstein-Barr virus (EBV) and herpes simplex virus type 1 (HSV-1), which likewise show increased antibody titers with higher stress (Esterling et al., 1993; Mcdade et al., 2000; Shirtcliff et al., 2009). Taken together, these studies provide preliminary evidence for an association between psychological stress and CMV reactivation; however further investigation of these associations in larger, non-clinical and/or working-aged populations is warranted.

Table 1.1: Psychological stress and CMV reactivation studies.

Study	Population	N	Measure of Stress	Measure of CMV reactivation	Main Finding
Appels et al. (2000)	Coronary artery disease patients	30	Depressive symptoms and vital exhaustion ^a	CMV antibody titers	Patients classified as exhausted (N=15) showed elevated CMV titers compared to non-exhausted patients
Bennett et al. (2012)	Older adults (64 yrs)	222	Depressive symptoms	CMV antibody titers	CMV antibody titers were not related to CES-D scores; however, for the EBV+CMV+ group, higher EBV antibody titers were related to higher CES-D scores, but not for the EBV+CMV- group
Fagundes et al. (2012)	Newly-diagnosed breast cancer patients (57 yrs)	158	Fatigue and depressive symptoms	CMV antibody titers	Higher CMV antibody titers were associated with a greater likelihood of being fatigued, but not depressive symptoms
Glaser et al. (1985)	First-year medical students	20	Academic examinations	CMV antibody titers	Found a significant increase in levels of CMV titers during the first day of exams as compared to a non-stress baseline (the end of summer vacation)
Jaremka et al. (2013)	Breast cancer survivors (51 yr)	200	Loneliness, depression, fatigue and pain	CMV antibody titers	Higher CMV antibody titers were associated with higher loneliness and levels of the symptom cluster (pain, depression, and fatigue)
Matalka et al. (2000)	CMV-positive female nursing students	12	Academic examinations	Defined as a 30% increase in virus-specific IgG levels in sera	Found CMV reactivation in 26% (N=12) of students during exams in both semesters; larger stress effects on CMV reactivation were found during the winter season

^a Vital exhaustion: a constellation of symptoms characterized by listlessness and fatigue

Table 1.2: Psychological stress and CMV reactivation studies (continued)

Study	Population	N	Measure of Stress	Measure of CMV reactivation	Main Finding
Mehta et al. (2000)	Astronauts	71	Space flight stress	Number of viral particles in urine using qPCR ^b and antibody titers	10.2% of the astronauts shed viral particles in urine samples around the time of their mission, compared to 1.2% of controls; among shedders, CMV IgG antibody titer increased from pre- to post-flight
Phillips et al. (2008)	Older adults (65+ yrs)	137	Anxiety & depression symptoms	CMV antibody titers	Higher CMV antibody was associated with higher depressive symptoms, anxiety and overall psycho morbidity
Sarid et al. (2004)	First-year nursing and physiotherapy students	54	Academic examinations	CMV-specific salivary IgG and IgA antibody	Found an approximate 65% increase in CMV-specific salivary IgG and a 46% increase in CMV-specific salivary IgA, compared to the beginning of the semester
Toro & Ossa (1996)	Healthy CMV-positive individuals	11	Stressful psychological and physical events ^c	CMV DNA by qPCR in blood, throat washings and urine	Found a positive association between CMV DNA and stress-producing events in the workplace over a 6-month period
Trzonkowski et al. (2004)	Patients with depression and controls	20	Depression	CMV antibody titers	Patients suffering from depression (N=10) had higher levels of CMV antibody, compared to healthy controls
Uddin et al. (2010)	Participants of the Detroit Neighborhood Health Study	100	Post-traumatic stress disorder (PTSD) ^d	CMV antibody titers	Those with PTSD (N=23) had an approximately 45% higher level of CMV-specific IgG antibody compared to unaffected individuals

^b qPCR: quantitative polymerase chain reaction; ^c Stressful events: physical trauma, surgery, X-ray or UV radiation exposure; academic tasks, work overload, insomnia; ^d PTSD: a severe anxiety disorder that can develop after exposure to a psychologically impactful event, like being a victim of crime, accident, or a disaster or being a close witness to such events

In addition to CMV reactivation, psychological stress may also impact the prevalence of CMV infection, although the evidence in this regard is mixed. For example, Miller and colleagues (2005) investigated the association between serostatus and depressive symptoms in sample of 65 patients recovering from an acute coronary syndrome. After splitting the subjects into three equal groups based on depression scores (using the Hamilton Rating Scale for Depression and Beck Depression Inventory), cardiac patients in the highest depression tertile were more likely to be CMV seropositive than the middle and lower tertiles. However, Phillips et al. (2008) found no association between CMV serostatus and depression and anxiety symptoms in 137 older adults.

Personality

Personality has been described as a characteristic manner in which one feels, thinks, behaves, and relates to others. As such, personality represents a powerful summary construct that encompasses the biological, experiential, and social aspects of an individual. Unlike other psychological constructs, such as mood or cognition, these characteristics are stable over time and, thus, reliably predict many aspects of health and longevity (reviewed in Chapman et al., 2011). Several models have been proposed to explain these links between personality and disease; however, many of these models have not been commonly tested (reviewed in Smith & Mackenzie, 2006). Despite the clear evidence for these associations, the underlying pathways remain to be elucidated.

Certain personality traits not only lead to increased occurrence of stressors, but can also affect the reactivity to stressors (Segerstrom, 2000). For example, Bolger and Schilling (1991) found that those high in neuroticism reported more exposures to daily stressors and experienced more distress from these situations, compared to those with low neuroticism.

Epidemiological data has, indeed, associated high levels of neuroticism with elevated evening levels of cortisol among subjects aged <75 years (Gerritsen et al., 2009), suggesting HPA-axis dysregulation in these individuals. This enhanced stress reactivity may form a basis for more frequent CMV reactivation among these individuals via the neuroendocrine pathways described above. Also, less well-considered is that personality also dictates several enduring behavioral features which may increase exposure to infectious disease, including sexual behaviors and social contacts (Asendorpf & Wilpers, 1998; Hoyle et al., 2000). The implication here is that stable personality traits could systematically predispose certain individuals to not only immune suppression, but also to more opportunities for infection. Thus, personality may be particularly relevant in the context of stress and immunosenescence.

Physical activity

Physical activity is simply defined as any body movement produced by skeletal muscular action that increases energy expenditure. Included under this umbrella term are more purposeful categories of movement, including sports, exercise and occupational and household activities (Caspersen et al., 1985). Meta-analyses have shown dose-response relationships of physical activity with 19% to 24% reductions in all-cause mortality rates for more active individuals (Woodcock et al., 2011). A physically active lifestyle is associated with a wide range of both physical and mental health benefits. How physical activity affects immune function and the risk of infection, however, remains under debate (Gleeson et al. 2012; Romeo et al., 2010; Walsh et al., 2011).

There is a strong contention that regular exercise can delay or even reverse features of immunosenescence (Drela et al., 2004; Kohut & Senchina, 2004; Simpson et al., 2012). For example, moderate intensity exercise training in mice has resulted in reduced numbers of

memory T-cells (Woods et al., 2003), while telomere-stabilizing proteins are elevated in trained mice, and humans, compared to their untrained counterparts (Werner et al., 2009). Importantly, increased aerobic fitness was associated with lower proportions of highly-differentiated T-cells in men (Spielmann et al., 2011). This association was unaffected by CMV infection status, implying that frequent exercise may counteract immunosenescence irrespective of infection with this immune-dominant virus. This finding may result from selective mobilization of highly-differentiated cells, including CMV-specific T-cells, into circulation with exercise, possibly leading to their subsequent deletion (Simpson, 2011).

However, in longitudinal studies, these types of immune benefits accompanying improved fitness are inconsistent at best. The variation in study designs (e.g., modes, durations, and intensities) employed by different researchers may be confounding the effect of physical activity in these studies (Simpson et al., 2012). There are hints that longer training regimens show more consistent immune effects, and combined with the larger fitness gap covered in cross-sectional studies, this observation suggests that the volume of participation in physical activity, in general, may enhance immunity later in life (Simpson & Bosch, 2014). It should also be considered that too much exercise (or other stressors) in early life (childhood to mid-twenties) may be detrimental to immunity later in life (Prieto-Hinojosa et al., 2014). Altogether, it appears that physical activity may improve immune functioning and resistance to infection by directly combating features of immunosenescence, such as the accumulation of highly-differentiated T-cells. This assumption is predicated on a long-term commitment to physical activity, which has not been examined in the context of T-cell differentiation to date.

Glucose metabolism

Metabolism, as referred to here, is the tightly regulated process of cellular utilization of bodily nutrients which provides energy. During the immune response to infection, large amounts of resources and energy (e.g., cell division, temperature elevation, cytokine production) are mobilized to effectively deal with the threat (Ganeshan & Chawla, 2014; Maciver et al., 2008; Pearce, 2010). A similar response is elicited for both physical and psychological threats, whereby anticipation of ‘fight or flight’, and potential bodily insult, results in the release of glucose and other metabolites into circulation. Glycated hemoglobin (HbA1c) is a long-term marker which captures the average circulating glucose levels over the previous 3-4 months in a single measurement (Peterson et al., 1998). Glucose metabolism is influenced by multiple behavioral factors, and, as such, elevated HbA1c levels have been associated with increased psychological stress (Schuck, 1998), certain personality types (Lane et al., 2000; Vollrath et al., 2007; Waller et al., 2013) and decreased physical activity (Umpierre et al., 2011).

In the very elderly (85 years old), elevated levels of HbA1c, fasting glucose and an increased prevalence of type-2 diabetes have been found among CMV+ individuals, compared to those CMV– (Chen et al., 2012). This elevated level of glucose may be a cause or consequence of CMV infection and/or reactivation. Dysregulation of glycemic control (e.g., chronic psychological stress and HPA-axis activation) can result in inappropriately elevated circulating glucose levels and increased production of reactive oxygen species (Cohen et al., 2011; Epel, 2009). This oxidative stress not only suppresses immunity, but its downstream by-products also may directly trigger CMV reactivation (Jaganjac et al., 2010; Muriach et al., 2008). So, whether considered independently, or in combination with CMV, chronically

elevated levels of glucose are associated with multiple features reminiscent of immunosenescence, including shorter telomere length and increased T-cell accumulation (Adaikalakoteswari et al., 2007; Maciver et al., 2008; Zhao et al., 2007). These hallmarks can even be seen at earlier stages of hyperglycemia and impaired glucose tolerance (Adaikalakoteswari et al., 2007; Salpea & Humphries, 2010) and are evident in poorer control and increased severity of infection in diabetic individuals (Allard et al., 2010; Joshi et al., 1999).

Conversely, CMV is known to hijack its host's metabolism to create an environment conducive to increased viral replication. Insulin-producing β -cells of the pancreas are permissive to CMV infection. Once infected, the resultant immunogenicity, ensuing inflammation and β -cell destruction may lead to decreased insulin production, thus elevating glucose levels and suppressing immunity (Smelt et al., 2012). It is possible that this CMV manipulation and the abovementioned oxidative stress synergize to accelerate the accumulation of highly-differentiated T-cells; however, the association between glycemic control and the accumulation of these cells has not been investigated directly.

Conclusion and the aim of this thesis

Herein is presented a model whereby infection history, particularly latent infection with and reactivation of CMV, is a putative shared mechanism linking psychosocial and behavioral factors and the age-related decline in immunity. Specifically, it is hypothesized that CMV may act as a mediator between psychological stress, personality, physical activity and glucose metabolism and immunosenescence (Figure 1.1). Thus, the aim of this thesis is to explore the validity of this hypothesis in a large sample of working-aged adults. Chapter 3 explores the association of multiple measures of psychological stress and the reactivation of

CMV, measured as levels of CMV-IgG antibody. Chapter 4 explores the influence of personality traits on CMV infection prevalence and CMV-IgG levels. Chapter 5 investigates the impact of physical activity frequency on the age-related accumulation of highly-differentiated T-cells, and the effects of CMV infection on these relationships. Chapter 6 explores the role of dysregulated glucose metabolism in the accumulation of highly-differentiated T-cells.

CHAPTER 2 : GENERAL METHODS

Study samples

The Mannheim Institute of Public Health (MIPH) Industrial Cohort Studies (MICS) perform voluntary health checks on the employees of an airplane manufacturing company in the south of Germany. Participants included in this thesis are from two separate sub-samples of the 2007 and 2011 collections. Only participants who gave informed consent were included in this thesis. There were no specific inclusion/exclusion criteria for participation in the health check. Questionnaire and physical examination data was available for N=910 and N=1454 individuals from 2007 and 2011, respectively. From 2007, N=887 (97%) of the subjects had plasma samples available for CMV antibody determination and were included in the analyses. From the 2011 collection, N=1103 (76%) of the subjects had a complete set immune data measured and were included in analyses. The participant characteristics are presented in Table 2.1. On average, participants were predominantly males in their early- to mid-forties. They were mostly married, non-managerial skilled workers, right below the upper limit for normal BMI and waist-to-hip ratio. Approximately one-fourth were current smokers, smoking less than a pack per day.

Table 2.1 Participant characteristics - MICS 2007 and 2011

	2007	2011
N	887	1103
CMV positive (%)	37	36
Age (years)	44.7 ± 10.1	40.1 ± 11.0
Gender (% male)	88	88
Married/Co-habiting (% yes)	70	77
Job status (%)	--	--
<i>Division/Dept manager</i>	4	4
<i>Project Leader/process mgr</i>	19	15
<i>Skilled worker (non-mgr)</i>	71	72
<i>Semi-skilled worker</i>	6	9
Current smoker (% yes)	26	29
Cigarettes per day (in smokers)	13 ± 12	14 ± 8
Body mass index (kg/m ²)	24.5 ± 3.5	24.5 ± 4.0
Waist-to-hip ratio	0.92 ± 0.07	0.90 ± 0.08

Values are mean ± SD unless otherwise specified

Measurements¹

The questionnaire and physical examination collection were performed by HealthVision Ltd. (Berlingen, Switzerland), subsequent biochemical analyses on collected samples were completed by SynLab (Leinfelden, Germany) and all the data for this thesis was provided via the MIPH. The set of measurements differed slightly between the two collections; thus, some variables were included in 2007, but not in 2011, and vice versa. The variables available from each respective collection are listed in Table 2.2. The most notable distinction lies in the CMV and T-cell differentiation data. In both samples, CMV serostatus (i.e., infection/not infected) was available. In the 2007 sample, I further analyzed continuous levels of CMV-specific antibodies. In the 2011 sample, I collected the immunological data for

¹ Parts of this general methods section are written in the first person to indicate where work was conducted by J. L. Rector rather than using pre-existing data from the rest of the epidemiological study

T-cell differentiation determination, but due to limited financial resources, was not able to further analyze plasma samples for CMV antibodies (see description below). The data presented in chapters 3 and 4 were drawn from the MICS 2007 dataset using CMV-IgG antibody levels as a measure of CMV reactivation. The data presented in chapters 5 and 6 were drawn from the MICS 2011 dataset using the accumulation of highly-differentiated T-cells as a measure of immunosenescence.

Table 2.2: Variable availability in the 2007 and 2011 MICS datasets

Variable	2007	2011	Variable	2007	2011
Demographic			Psychological variables		
<i>Age</i>	✓	✓	<i>Anxiety (HADS)</i>	✓	✗
<i>Gender</i>	✓	✓	<i>Depression (HADS)</i>	✓	✗
<i>Marital status</i>	✓	✓	<i>Vital exhaustion(MQ)</i>	✓	✓
			<i>Subjective mental health (SF-12)</i>	✓	✓
Socioeconomic			Metabolic factors		
<i>Education</i>	✓	✗	<i>HbA1c</i>	✓	✓
<i>Job status</i>	✓	✓	<i>Fasting glucose</i>	non-fasting	✓
<i>Income</i>	✓	✗	<i>Total cholesterol</i>	✓	✓
<i>Manual occupation</i>	✗	✓	<i>Low density lipoprotein (LDL)</i>	✓	✓
<i>Shift worker</i>	✗	✓	<i>High density lipoprotein (HDL)</i>	✓	✓
			<i>Triglycerides</i>	✓	✓
Lifestyle			Biological factors		
<i>Current smoking</i>	✓	✓	<i>C-reactive protein (hs-CRP)</i>	✓	✓
<i>Cigarettes per day</i>	✓	✓	<i>Fibrinogen</i>	✓	✗
<i>Alcohol intake</i>	continuous	categorical	<i>Metabolic syndrome</i>	✗	✓
<i>Body mass index</i>	✓	✓	<i>Resting heart rate</i>	✓	✓
<i>Waist-to-hip ratio</i>	✓	✓	<i>Blood pressure</i>	✓	✓
<i>Waist circumference</i>	✓	✓			
<i>Physical activity (kcal/day)</i>	✓	✗	Immune parameters		
<i>Leisure activity (h/wk)</i>	✗	✓	<i>T-cell phenotypes</i>	✗	✓
			<i>CMV status</i>	✓	✓
Big 5 personality traits	✓	✗	<i>CMV-specific antibodies (IgG)</i>	✓	✗
Physical activity frequency	✗	✓			

✓ = available; ✗ = unavailable; Further details for each variable is available in the relevant methods sections of the chapters in this thesis.

Cytomegalovirus (IgG) serostatus determination

For both the 2007 and 2011 samples (N=2,341 total), fasting plasma samples were stored in small aliquots at -80°C until analysis. Evidence of previous CMV infection (serostatus) was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (BioCheck, Inc., CA, USA) according to manufacturer instructions. Optical density values obtained from participant samples were fitted to a standard curve. These concentrations were then compared to a cut-off value to compute CMV index scores. Participants with a borderline seropositive result, representing a calculated index score >0.85 and <1.15 , were retested (N=16 in 2007; N=9 in 2011). If they remained borderline, subjects with index scores above and below 1.00 were considered positive (CMV+) and negative (CMV-) respectively, as per manufacturer instructions. The sensitivity, specificity, and accuracy of the test are reported as 95.0, 96.7 and 96.0%, respectively.

For the 2007 subjects only, I further analyzed the plasma samples for continuous levels of CMV-IgG among those infected. Because the BioCheck assay is not recommended for quantitative determination of antibody levels, I measured CMV-IgG with a commercially available ELISA kit (Genesis Diagnostics Ltd., Cambridgeshire, UK) according to the manufacturer's instructions. The manufacturer's reported within-assay and between assay imprecision was $<12\%$. Like the BioCheck assay, each subject was assayed in duplicate and the scores averaged to yield the final antibody concentration. These concentrations were used in subsequent analyses.

Flow cytometry

During the 2011 collection only, I prepared the subject samples, on-site, for T-cell phenotyping by flow cytometry. Whole blood samples were collected by trained nurses in

ethylenediaminetetraacetic acid (EDTA) coated tubes (Sarstedt, Nümbrecht, Germany). These samples were stored at room temperature and I prepared them within 1 hour of collection. Upon receiving the sample, I stained 30 μ L of the whole blood with a combination of the following monoclonal antibodies: CD3 APC-Cy7, CD4 PerCP, $\gamma\delta$ TCR PE, CD8 APC, (BD Biosciences, San José, CA), CD45RA FITC, and CD27 PE-Cy7 (BD Pharmingen, San Diego, CA). Following 20 minutes of incubation at room temperature in the dark, I added 1.5 mL of BD FACS lysing solution to the mixture, which was incubated for another 15 minutes. After a 7-minute centrifugation at 700xg, I removed the supernatant and re-suspended the pellet in 250 μ L of 2% paraformaldehyde solution until analysis. Data was collected using a FACSCanto II flow cytometer (BD Biosciences, San José, CA) and the FACS Diva software (BD Biosciences, San José, CA) with at least 10,000 gated lymphocyte events collected for each tube. The cytometer was regularly calibrated by laboratory personnel. Spectral overlap was electronically compensated for using single labelled antibody tubes.

Following acquisition, FCS files were transferred to a third party software program (FlowJo v7.6.5, Tree Star, Inc., Ashland, OR) for analysis. Figure 2.1 shows representative plots of the gating strategy employed for each subject file. Briefly, gated CD3⁺ (T-lymphocytes) were stratified by $\gamma\delta$ TCR expression. Both subsets of CD3⁺ T-cells, i.e., $\gamma\delta$ TCR⁺ and $\gamma\delta$ TCR⁻ (referred to from here on simply as CD3⁺ T-cells), were further subdivided by their expression of CD4 and CD8. Single-positive CD8⁺ T-cells (cytotoxic T lymphocytes; CTL) were then split into stages of differentiation based on their CD27 and CD45RA expression. CD27⁺CD45RA⁺ were naive (NA), CD27⁺CD45RA⁻ were central memory (CM), CD27⁻CD45RA⁻ were effector memory (EM), and CD27⁻CD45RA⁺ were CD45RA re-expressing effector memory (EMRA). The same CD27/CD45RA classification

was made with $\gamma\delta$ TCR⁺ T-cells. These percentages were multiplied by absolute numbers of lymphocytes to obtain cell numbers. The $\gamma\delta$ TCR⁺ subset were predominantly CD4 and CD8 double-negative or CD8 single-positive, and because of their low frequency in peripheral blood, these subsets were added together and simply analyzed as total NA, CM, EM, and EMRA $\gamma\delta$ TCR⁺ T-cells.

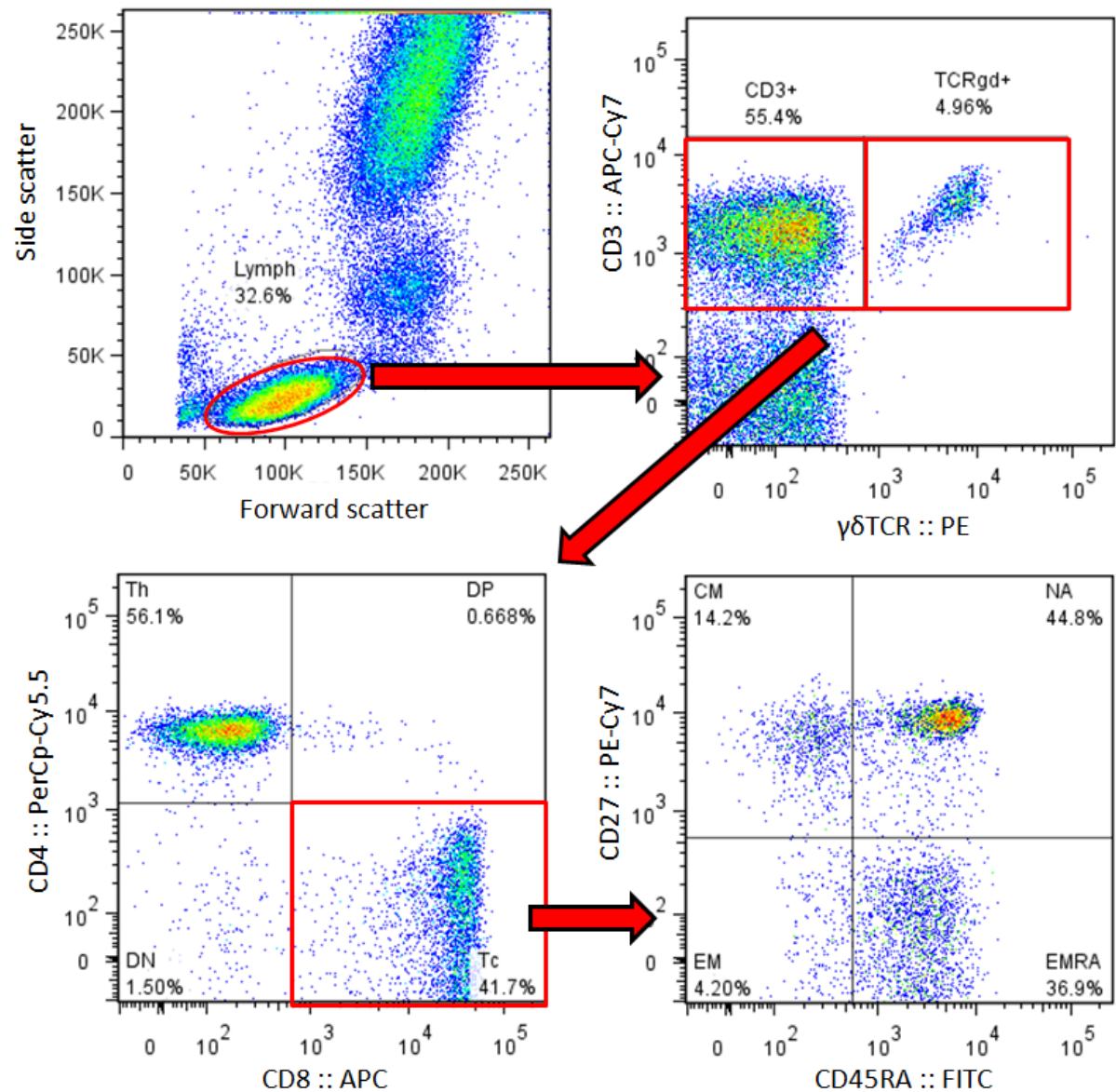


Figure 2.1: Sample gating strategy to derive T-cell subsets.
 Lymph, lymphocytes; CD, cluster of differentiation; TCRgd, gamma-delta ($\gamma\delta$) T-cell receptor; Th, CD4+ helper T-cell; Tc, CD8+ cytotoxic T-cell; DP, double-positive CD4/CD8; DN, double-negative CD4/CD8; NA, naive; CM, central memory; EM, effector memory; EMRA, CD45RA+ effector memory.

Data collection and analyses

The MIPH sent the 2007 samples to the University of Birmingham, where I independently completed the BioCheck and Genesis CMV IgG antibody ELISA analyses in the Behavioral Immunology laboratory.

I set up and worked in the on-site laboratory for the 2011 collection in Augsburg, Germany. The above-described preparation of participant samples for flow cytometry, and the collection of plasma for later CMV serological testing, were carried out by an on-site laboratory assistant (S. Stark) and I. The daily collection of flow cytometry data was carried out by two laboratory assistants at the MIPH (I. Gregei & T. Rausch) and me. After the collection, the frozen plasma samples were sent from the MIPH to the University of Birmingham, where I carried out the ELISA analyses with the laboratory assistance of L. Rotar. Ms. Rotar also assisted with the analysis of the flow cytometry data.

CHAPTER 3 : CONSISTENT ASSOCIATIONS BETWEEN MEASURES OF PSYCHOLOGICAL STRESS AND CMV ANTIBODY LEVELS IN A LARGE OCCUPATIONAL SAMPLE

Abstract

Cytomegalovirus (CMV) is a herpes virus that has been implicated in biological aging and impaired health. Evidence, largely accrued from small-scale studies involving select populations, suggests that stress may promote non-clinical reactivation of this virus. However, absent is evidence from larger studies, which allow better statistical adjustment for confounding and mediating factors, in more representative samples.

The present study involved a large occupational cohort (n=887, mean age=44, 88% male). Questionnaires assessed psychological (i.e., depression, anxiety, vital exhaustion, SF-12 mental health), demographic, socioeconomic (SES), and lifestyle variables. Plasma samples were analyzed for both the presence and level of CMV-specific IgG antibodies (CMV-IgG), used as markers for infection status and viral reactivation, respectively. Also assessed were potential biological mediators of stress-induced reactivation, such as inflammation (C-reactive protein) and HPA function (awakening and diurnal cortisol). Predictors of CMV infection and CMV-IgG among the infected individuals were analyzed using logistic and linear regression analyses, respectively.

Confirming prior reports, lower SES (education and job status) was positively associated with infection status. Among those infected (N=329), higher CMV-IgG were associated with increased anxiety ($\beta=.14$, $p<.05$), depression ($\beta=.11$, $p=.06$), vital exhaustion

($\beta=.14$, $p<.05$), and decreased SF-12 mental health ($\beta=-.14$, $p<.05$), adjusting for a range of potential confounders. Exploratory analyses showed that these associations were generally stronger in low SES individuals. We found no evidence that elevated inflammation or HPA-function mediated any of the associations.

In the largest study to date, we established associations between CMV-IgG levels and multiple indicators of psychological stress. These results demonstrate the robustness of prior findings, and extend these to a general working population. We propose that stress-induced CMV replication warrants further research as a psychobiological mechanism linking stress, aging and health.

Introduction

There is convincing evidence that psychological stress impacts health, with the immune system likely playing an important mediating role (Miller et al., 2009; Segerstrom & Miller, 2004). An elegant *in vivo* paradigm to study the impact of stress on the immune system is the reactivation of latent herpes viruses, such as herpes simplex virus (HSV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), or cytomegalovirus (CMV) (Glaser & Kiecolt-Glaser, 1997; Glaser & Kiecolt-Glaser, 2005). These infections are distinctive because the host is unable to completely eliminate the virus, establishing a life-long competition between the pathogen and the host immune system (Sinclair, 2008). In immune competent individuals, the virus mostly remains in a dormant (i.e., low-replicating) state, denoted as latency. However, when immune control is weakened, the virus begins to replicate, which in turn stimulates memory B lymphocytes to increase the output of virus-specific IgG antibody. This increase results in the seemingly paradoxical observation that higher antibody levels reflects poorer immune control of the virus (Glaser & Kiecolt-Glaser, 1994; Kuo et al., 2008; Van Zanten et al., 1995).

The current study focused on psychosocial factors related to CMV infection status and CMV-IgG levels (reflecting reactivation of the virus). CMV is a highly prevalent β -herpes virus which asymptomatically infects between 30% and 90% of the population in developed countries (Staras et al., 2006). Prevalence of CMV increases nearly linearly with age (Crough & Khanna, 2009; Staras et al., 2006) and with lower socioeconomic status (SES) (Dowd et al., 2009; Enders et al., 2012; Mustakangas et al., 2000; Simanek et al., 2009). CMV has long been considered harmless to healthy immune competent hosts. More recently, this consensus has been revised on the basis of studies that have associated this virus with increased

mortality, especially among older adults (Gkrania-Klotsas et al., 2012; Pawelec et al., 2012; Simanek et al., 2011; Strandberg et al., 2009). These epidemiological findings are complemented by studies showing correlations between CMV infection and poor health outcomes, such as the development of metabolic and cardiovascular diseases (Cheng et al., 2009; Haarala et al., 2012; Hjelmestaeth et al., 2004; Nabipour et al., 2006), autoimmune disease (Söderberg-Nauclér, 2012; Varani & Landini, 2011) some cancers (Dziurzynski et al., 2012; Michaelis et al., 2009), as well as cognitive decline and poor functional status (Aiello et al., 2006; Gow et al., 2013; Moro-Garcia et al., 2012).

One prominent explanation for these health effects pertains to the possible role of CMV in accelerating aging of the immune system, a process denoted as immunosenescence (Pawelec et al., 2009; Turner et al., 2014). Indeed, studies show that CMV infection and CMV-IgG levels are associated with markers of impaired immunity that characterize aging. These include impaired vaccination responses (Mcelhaney et al., 2012; Turner et al., 2014), increased inflammation (Freeman, 2009; Qiu et al., 2008), selective accumulation of T-lymphocytes with impaired responsiveness to mitogens (Chidrawar et al., 2009), reduced telomere length (Van De Berg et al., 2010), and reduced telomerase activity (Dowd et al., 2013). Together these findings suggest that research identifying factors that predict CMV infection status and reactivation may significantly contribute to understanding the determinants of healthy aging (Nikolich-Zugich, 2008).

Psychological stress has been identified as one of the factors that can drive subclinical CMV replication, representing a potential mechanism linking stress, immunity and aging (Bosch et al., 2012). In one of the earliest studies, Lycke et al. (1974) found that hospital psychiatric patients had higher CMV-IgG than healthy controls. Subsequent confirmations

were provided by naturalistic stress studies involving caregiving (Pariante et al., 1997), spaceflight (Mehta et al., 2000), academic exams (Glaser et al., 1985; Matalka et al., 2000; Sarid et al., 2004), post-traumatic stress disorder (PTSD) (Uddin et al., 2010), and childhood adversity (Dowd et al., 2012; Fagundes et al., 2013). Studies assessing self-reported stress confirmed these associations, and helped to further characterize the psychological variables involved. For example, studies in older adults identified depression and anxiety as factors associated with higher CMV-IgG (Phillips et al., 2008; Trzonkowski et al., 2004). In a cohort of cardiovascular patients, Appels, Bär, et al. (2000) found higher CMV antibody levels among those reporting vital exhaustion (VE), a state characterized by lack of energy, increased irritability, and feelings of demoralization (Appels, Kop, et al., 2000; Appels & Mulder, 1988). Related, higher levels of fatigue were associated with higher CMV-IgG in breast cancer patients (Fagundes et al., 2012).

Despite apparently consistent associations between stress and CMV reactivation, the interpretation of these findings is hampered by some limitations. First, many of the aforementioned studies allowed only limited statistical adjustments due to very small sample sizes². This leaves ambiguity with regard to the proper interpretation of these findings (e.g., potential confounding). A second limitation is that prior reports involved samples drawn from select populations, like patient groups, astronauts, or older adults, which creates uncertainty with regards to the generalizability of the findings. To determine the robustness and generalizability of the observed associations between psychological factors and impaired viral control, research in larger and more representative populations would be needed (Dowd et al., 2008).

² To date only 4 studies had an N>50, two of which involved cancer patients or survivors (Fagundes et al. 2012; Jaremka et al. 2012) two studied older adults (Bennett et al. 2012; Phillips et al. 2008).

A further limitation of the extant literature is that little attention has been paid to possible intermediate mechanisms. For example, experimental studies show that inflammatory mediators (Döcke et al., 1994; Fagundes et al., 2012; Stein, Volk, Liebenthal, Krüger, et al., 1993) and glucocorticoids (Lathey & Spector, 1991; Tanaka, Ogura, Kamiya, Sato, et al., 1984) can promote CMV reactivation. As these factors may also become elevated in response to psychological stress, these represent indirect pathways linking stress and CMV reactivation (cf. Fagundes et al., 2012). In order to address these limitations, the present study investigated associations between CMV reactivation, anxiety, depression, and vital exhaustion in a large sample of working adults. Analyses involved adjustment for demographic factors and health behaviors, and also tested if inflammation and HPA activity may act as possible mediators. Informed by prior research showing a larger impact of stress in low-SES individuals (Brydon et al., 2004; Gruenewald et al., 2006), we also performed exploratory analyses to determine if associations between psychological predictors and CMV-IgG may vary by SES group.

Materials and Methods

Participants

The present study was conducted in a cross-sectional sample of the Mannheim Institute of Public Health Industrial Cohort Studies (MICS), consisting of 887 employees who took part in a voluntary company health check (Herr et al., 2012; Li et al., 2013). Participant characteristics are presented in Table 3.1. Participants received a personalized health report. All data was anonymized before analysis. The study was approved by the Ethical Committee of the Mannheim Medical Faculty, Heidelberg University, and all participants signed informed consent.

Procedures

Participants arrived in the morning between 06:45h and 08:45h for assessment. After a fasting blood draw, participants were seated separately in a quiet room to fill out questionnaires at a location away from their usual workplace. Demographic, medical, and health behavior data were assessed by questionnaire, anthropometric measurements (e.g., height, weight, waist and hip circumference) and blood pressure were determined by trained personnel, using standard procedures.

Psychological questionnaires and quality of life

Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983), which is commonly used in general population studies (Crawford et al., 2001; Mykletun et al., 2001). The anxiety and depression subscales comprise 7 items using a 4-point Likert scale format (Cronbach's α of .81 and .82, respectively). The 9-item Shortened Maastricht Exhaustion Questionnaire (MQ) measured vital exhaustion (VE), which is reflective of lack of energy, increased irritability, and feelings of demoralization (Kopp et al., 1998). Higher scores indicate increased vital exhaustion (Cronbach's α =.91). The 12-item Health Survey (SF-12), version 2, evaluated subjective quality of life on two dimensions – physical and mental health. For the purpose of this study only the latter subscale was analyzed. Higher scores (range 0-100) indicate better well-being (Ware et al., 1996). Sleep quality was measured by the Jenkins Sleep Problems Scale (Jenkins et al., 1988), which assessed the frequency of four common sleep disturbances (6-point Likert scale) within the last month (ranging from “not all” to “22-31 nights”), including trouble falling asleep, waking up at night, trouble staying asleep, and waking feeling tired and worn out (Cronbach's α =.79).

Socio-demographic and lifestyle data

Demographic data (e.g., age, gender, and marital status), SES indicators (job status, education level, and net monthly income), and health behaviors (e.g., smoking, alcohol, physical activity, and diet) were obtained by questionnaires previously validated in the MONICA study (Jönsson et al., 1999). Participants were identified as current smokers by self-report and smoking frequency was assessed as the number of cigarettes smoked per day. Alcohol consumption was assessed as the cumulative number of alcoholic beverages, specified in mean grams of alcohol per week.

Cytomegalovirus (CMV) antibody levels

Fasted blood samples were taken by venipuncture in ethylenediaminetetraacetic acid (EDTA) coated tubes (Vacutainer, BD, Plymouth, UK). Plasma was obtained by centrifugation and stored in small aliquots at -80°C until analysis. Evidence of CMV infection (serostatus) was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (BioCheck, Inc., CA, USA) according to manufacturer instructions. Participants with a borderline seropositive result, classified as a calculated index score >0.85 and <1.15 , were retested. If they remained borderline (N=16), subjects with index scores above and below 1.00 were considered positive (CMV+) and negative (CMV-) respectively, which is consistent with manufacturer instructions. The sensitivity, specificity, and accuracy of the test were reported as 95.0%, 96.7%, and 96.0%, respectively. Because the BioCheck assay is not recommended for quantitative determination of antibody levels, CMV-IgG levels in CMV+ individuals were again measured by a commercially available ELISA kit (Genesis Diagnostics Ltd., Cambridgeshire, UK) according to the manufacturer's instructions. The company-reported within-assay and between assay imprecision was $<12\%$.

Inflammation

Analysis of high sensitivity C-reactive protein (hs-CRP) was performed by immunonephelometry using a Behring Nephelometer II (High Sensitivity CRP, Dade Behring). The detection limit for hs-CRP was 0.015 mg/L, with intra-assay and inter-assay CV% < 10%. All analyses were done at Synlab (Leinfelden, Germany).

HPA activity

Salivary cortisol was analyzed using a cortisol luminescence immunoassay (sensitivity=.008 µg/dL; intra-CV%≤4.5%; IBL International GmbH, Hamburg, Germany) and were performed at the lab of Prof. Kirschbaum, at the Dept. of Biopsychology, TU Dresden, Germany. To obtain a cortisol awakening response (CAR) participants were instructed to collect saliva immediately upon awakening and 30 minutes after awakening (Wüst et al., 2000) using time-labeled saliva collection tubes (polyester salivette, Sarstedt, Germany). The participants were provided with a diary to record the exact collection times. Further samples were collected at 2 hours post-awakening, at 18:00h, and at 22:00h to calculate diurnal cortisol area under the curve (AUC) (Golden et al., 2011). Participants were instructed to store the saliva samples in their refrigerator or freezer overnight. On return, all samples were immediately frozen at –20 °C until assay (within 8 weeks).

Statistical analysis

First, *t*-tests and χ^2 analyses were used to test for differences in covariates of interest between CMV+ and CMV– individuals. Binary logistic regression was used to calculate odds ratios (OR) and corresponding 95% CIs of CMV infection, adjusting for age and gender. CMV-IgG levels (among CMV+ individuals) were log-transformed to approximate a normal

distribution. Linear regression analyses were then conducted to test the associations of socio-demographic and psychological stress measures with CMV-IgG levels. Potential confounders (e.g., age, gender, demographics, lifestyle, SES) were entered as covariates. Additional adjustments were performed to test cortisol and hs-CRP (log-transformed), as potential mediators of the psychological stress-CMV-IgG relationship. The CAR was calculated as the difference between the cortisol value at awakening and the value 30 minutes after awakening (Kunz-Ebrecht, Kirschbaum, Marmot, et al., 2004). Diurnal cortisol AUC was calculated according to the trapezoid rule described elsewhere (Pruessner et al., 2003). Lastly, analyses were stratified by education level and job status to test for SES differences in immune response to psychological stress. Analyses were carried out with SPSS version 19 (IBM-SPSS, Chicago, IL, USA).

Results

Predictors of CMV infection status

As shown in Table 3.1, 329 (37%) participants were CMV+. On average, CMV+ individuals were older, more likely to be married, and had lower education and job status. CMV+ individuals were also more likely to be current smokers, but infection status was unrelated to the quantity smoked. These associations remained significant after adjustment for age and gender (Table 3.2). Other lifestyle-related factors, such as alcohol intake, BMI, and activity levels were not associated with CMV infection status. Measures of psychological stress, including anxiety, depression, vital exhaustion, and SF-12 mental health scores were also not associated with CMV infection (see Table 3.2).

Predictors of CMV-specific IgG antibody levels

Increased HADS anxiety and depression, vital exhaustion, and lower SF-12 mental health scores were all significantly associated with increased CMV-IgG in CMV+ individuals, adjusting for age and gender (Table 3.3). After subsequent adjustment for predictors of CMV infection status (i.e., marital status, job status, education, smoking), these associations remained significant with the exception of depression, which was slightly attenuated ($\beta=.108$, $p=.06$) (see Table 3.3). For illustrative purposes, Figure 3.1 presents the average scores for each of these psychological parameters by three antibody tertiles (low, middle, and high CMV-IgG), showing a near-linear trend for all parameters.

Notably, none of the factors that predicted serostatus (i.e., education, job status, marital status, or smoking) were predictors of CMV-IgG level (Table 3.3). Other lifestyle factors (i.e., alcohol intake, BMI, waist-to-hip ratio, and activity level) were likewise not significantly associated with CMV-IgG level (all $p>.10$; analyses not shown).

Mediation by inflammation and HPA activity

In light of evidence from *in vitro* and clinical studies, we aimed to determine if cortisol levels and inflammation were 1) associated with CMV reactivation, and 2) might act as potential pathways linking stress and CMV reactivation. We found no evidence that cortisol levels, either measured as cumulative daily cortisol output (AUC), or CAR, was associated with CMV infection or reactivation (all $p>.10$). Similarly, systemic inflammation, measured as serum hs-CRP, was not significantly associated with infection status or CMV-IgG. Unsurprising therefore, when HPA parameters and hs-CRP were added to the multivariate model, the association between psychological variables and CMV-IgG was unaffected.

Exploratory analyses: SES moderates the associations between psychosocial stress and CMV-IgG level

Table 3.4 presents analyses of the relationship between psychological predictors (anxiety, depression, vital exhaustion, SF-12 mental health) and CMV-IgG stratified by education and job status. Linear regressions revealed substantially larger associations among those with lower education (apprenticeship or less) or lower job status (no managerial responsibility), which only in these groups reached statistical significance (adjustment for age and gender). However, formal tests of group x stress interaction did not reach significance (for all $p > .10$).

Table 3.1: Participant characteristics.

	Overall (N=887)	CMV Status		p
		CMV Positive N= 329 (37%)	CMV Negative N= 558 (63 %)	
Age (years)	44.7 ± 10.1	46.6 ± 9.8	43.6 ± 10.1	<.001
Gender (% male)	88	86	89	.240
Current smoker (% yes)	26	32	23	.003
Cigarettes/day (in smokers)	13 ± 12	14 ± 11	13 ± 12	.729
Alcohol consumption (g/day)	19.7 ± 27.9	20.3 ± 26.2	19.3 ± 28.8	.592
BMI (kg/m ²)	24.5 ± 3.5	24.5 ± 3.6	24.6 ± 3.5	.657
Waist-to-hip ratio	0.92 ± 0.07	0.92 ± 0.07	0.92 ± 0.07	.621
Activity (kcal/day)	475.4 ± 478.9	467.3 ± 442.1	480.2 ± 499.7	.703
Married (%)	70	76	66	.003
Education (%)				.001
<i>College/University</i>	15	12	17	--
<i>Vocational training</i>	16	18	15	--
<i>Completed apprenticeship</i>	66	65	67	--
<i>No higher education</i>	3	5	1	--
Job status (%)				.009
<i>Division/Dept manager</i>	4	4	5	--
<i>Project Leader/process mgr</i>	19	17	20	--
<i>Skilled worker (non-mgr)</i>	71	70	72	--
<i>Semi-skilled worker</i>	6	9	4	--
Income (%)				.979
<i>> 4000 €/mo</i>	10	9	10	--
<i>2500 - 4000 €/mo</i>	40	41	40	--
<i>1500 - 2500 €/mo</i>	44	44	44	--
<i>< 1500 €/mo</i>	7	6	7	--

All are unadjusted comparisons of participant characteristics. A *t*-test was performed on continuous variables and a χ^2 for categorical variables. Values are mean ± SD unless otherwise stated.

Table 3.2: Odds ratios (ORs) for socio-demographic and psychological stress factors with CMV infection.

	% CMV+	OR (95% CI)*	P_{trend}
Socio-demographic factors			
Education			.030
<i>College/University</i>	30	1.00 (Reference)	
<i>Vocational training</i>	41	1.47 (0.87 - 2.49)	
<i>Completed apprenticeship</i>	38	1.41 (0.92 - 2.15)	
<i>No higher education</i>	75	5.92 (1.75 - 20.06)	
Job status			.030
<i>Division/Dept manager</i>	32	1.00 (Reference)	
<i>Project Leader/process manager</i>	34	1.03 (.477 - 2.23)	
<i>Skilled worker (non-managerial)</i>	36	1.26 (.616 - 2.58)	
<i>Semi-skilled worker</i>	60	3.05 (1.18 - 7.85)	
Income			.908
> 4000 €/mo	36	1.00 (Reference)	
2500 - 4000 €/mo	38	1.18 (.687 - 2.02)	
1500 - 2500 €/mo	37	1.22 (.715 - 2.09)	
< 1500 €/mo	36	1.15 (.517 - 2.55)	
Marital status			.029
<i>Single (incl. divorced & widowed)</i>	30	1.00 (Reference)	
<i>Married</i>	41	1.49 (1.04 - 2.14)	
Smoking			.001
<i>No</i>	34	1.00 (Reference)	
<i>Yes</i>	46	1.79 (1.29 - 2.49)	
Psychological stress factors			
Depression (HADS)	--	1.05 (.912 - 1.21)	.503
Anxiety (HADS)	--	1.07 (.929 - 1.23)	.346
Mental health score (SF-12)	--	.925 (.804 - 1.07)	.283
Vital exhaustion (MQ)	--	1.03 (.893 - 1.19)	.688
Sleep disturbances (Jenkins)	--	1.07 (.927 - 1.23)	.356

Adjusted for age and gender. Psychological stress factors are standardized Z-scores. *95% confidence interval.

Table 3.3: Standardized regression coefficients for the association of socio-demographic and psychological stress factors with CMV-IgG levels within CMV+ individuals (n=329).

	Unadjusted	Model 1	Model 2
Socio-demographic factors			
Education	-.047	-.044	--
Job status	-.012	-.042	--
Income	-.085	-.056	--
Marital status (single or married)	-.055	-.012	--
Smoking (yes/no)	.104	.102	--
Psychological stress factors			
Depression (HADS)	.095	.109*	.108†
Anxiety (HADS)	.137**	.140**	.135*
Mental health score (SF-12)	-.149**	-.141**	-.137*
Vital exhaustion (MQ)	.148**	.146**	.142*
Sleep disturbances (Jenkins)	.145**	.145**	.136*

Model 1: Adjusted for age and gender; Model 2: Model 1 with additional adjustment for education, job status, marital status, and smoking. Significant at * $p < .05$, ** $p < .01$, † $p = .06$.

Table 3.4: Standardized regression coefficients for psychological factors and CMV-IgG levels by education level and job status.

	Low Education ^a (N=220)	High Education (N=93)	Low Job Status ^b (N=246)	High Job Status (N=65)
Depression (HADS)	.144*	-.067	.104	-.022
Anxiety (HADS)	.113	.111	.135*	.115
Mental health score (SF-12)	-.176**	-.086	-.141*	-.219
Vital exhaustion (MQ)	.142*	-.014	.110	.191
Sleep disturbances (Jenkins)	.181**	.006	.142*	.155

^a Low education is apprenticeship or no completed higher education; high education is completed vocational training or university. Missing (n=16). ^b Low job status is without managerial responsibility; high job status is with managerial responsibility. Missing (n=18). All analyses were adjusted for age and gender. Significant at * $p < .05$ & ** $p < .01$.

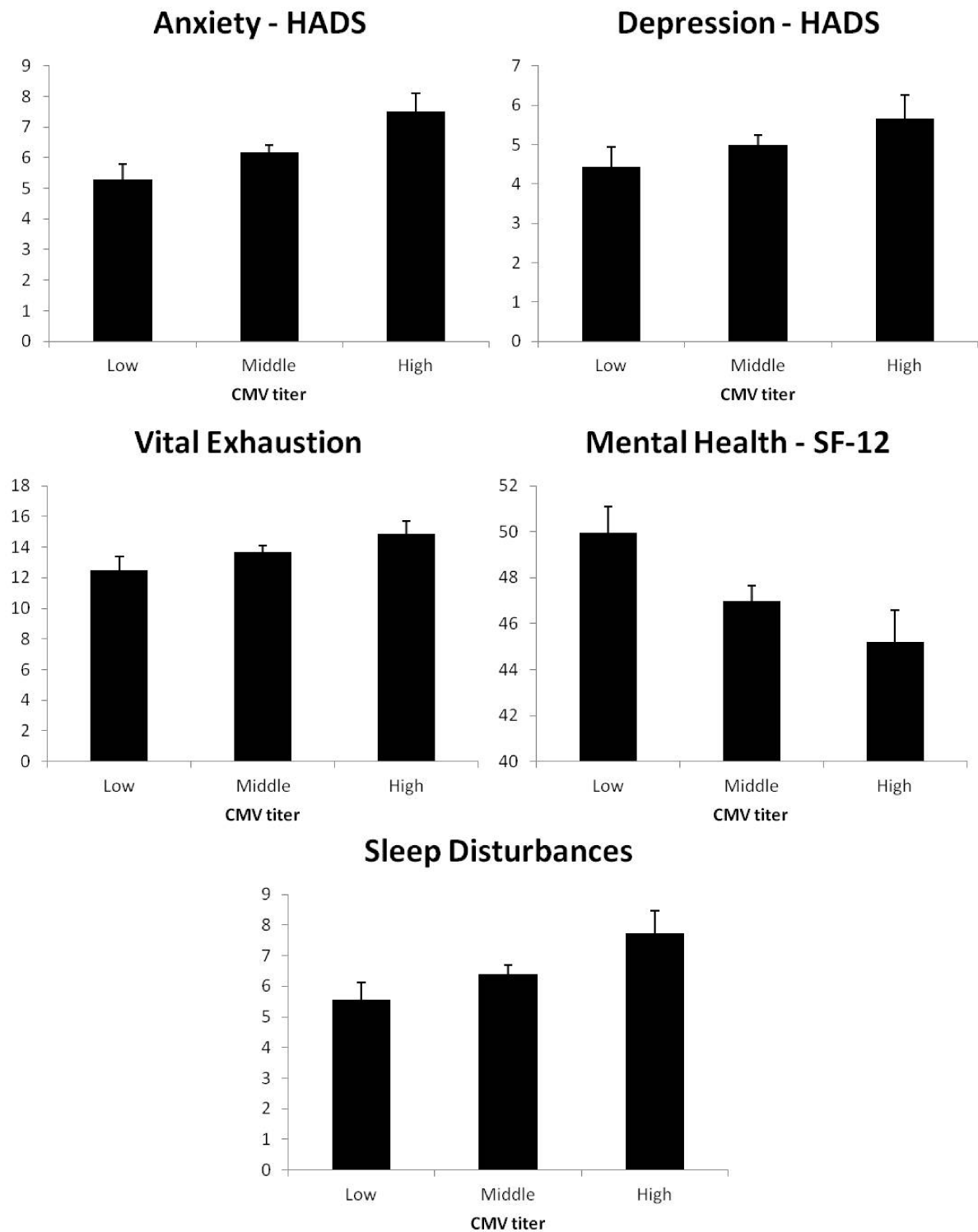


Figure 3.1: Mental health variables by three tertiles of CMV-IgG levels among CMV+ (n=329). Bars indicate means and standard error of the mean.

Discussion

CMV reactivation has been associated with impaired health and identified as a possible causal factor in the age-related decline of immune function. Psychological stress may be a determinant of CMV reactivation. The present study revealed consistent associations between measures of psychological stress and CMV-IgG in a large occupational sample. Higher anxiety, depression, vital exhaustion, and lower subjective mental health were all associated with elevated CMV-IgG levels in CMV+ individuals, but these factors were not associated with being infected *per se*. In contrast, lower SES was associated with increased odds of infection, but not with CMV-IgG levels. These associations withstood adjustment for a range of lifestyle and demographic variables. Overall, then, these findings suggest that stress-induced CMV reactivation is a robust phenomenon that can be readily replicated in a general population sample, extending what is known for other herpes viruses (e.g., HSV, EBV) to a species which has increasingly become a cause of public health concern (Gkrania-Klotsas et al., 2012; Roberts et al., 2010; Strandberg et al., 2009).

As noted by others (e.g., Aiello et al., 2009; Fagundes et al., 2012), most prior studies in this area lacked a systematic assessment of well-being and stress (or, at least, did not present the data). The current study utilized several validated measures, and the consistency across measures is therefore a novel finding in its own right. This consistency contributes to the recurrent debate on whether specific psychological factors or a general “negative affectivity” factor are better able to account for the health effects of stress (Cohen et al., 1995; Joiner et al., 1996; Marsland et al., 2001). While both viewpoints have received ample support, the results presented here appear more in line with the latter. It is also conceivable that a specific component of the shared variance, other than or in addition to negative affect, is

driving the observed associations. For example, there is substantial overlap observed between the vital aspects of depressive symptomatology and VE (Vroege et al., 2012) with depression reported as responsible for 58% of the variability in VE (Tselebis et al., 2011). Further, this hypothesis is consistent with a number of studies linking somatic/vital symptoms (e.g., fatigue, bodily signs) rather than cognitive and affective complaints (e.g., low mood, worrying) of anxiety and depression with inflammation, poorer health outcomes, and mortality (Duivis et al., 2013; Roest et al., 2013).

The current literature on psychosocial factors and CMV reactivation hinges on the assumption that CMV-IgG levels are reflective of viral activity. While this assumption has received empirical verification (Besson et al., 2006; Kuo et al., 2008; Van Zanten et al., 1995), and is consistent with what has been established for other herpesviridae (e.g., EBV, HSV), there are additional factors that may affect virus-specific antibody levels. For example, repeated infection with different strains of the same virus (denoted as super-infection) may likewise elevate CMV-IgG levels (Novak et al., 2008; Ross et al., 2010). In light of the pattern of results, super-infection would seem less likely as an alternative explanation: if elevated antibody levels reflected repeated infection, it can logically be expected that at least some of the factors that predicted infection (e.g., SES, marital status) would also predict CMV-IgG levels. That was not the case here. While it is conceivable that other factors, like genetic background, may co-determine antibody levels, and thereby confound the observed association, we are not aware of any research identifying such extraneous factors. Hence, reactivation is, at present, the most likely explanation for elevated CMV-IgG levels in distressed individuals (Glaser & Kiecolt-Glaser, 1994; Kuo et al., 2008). Future longitudinal studies linking temporal changes in mood and distress with fluctuation in CMV-IgG may

further corroborate this idea (cf. Faulkner & Smith, 2009; Stowe et al., 2007; Strachan et al., 2011).

The results showed that the association between CMV-IgG and psychological stress was strongest in low-SES individuals (job status, education), and virtually absent in high-SES employees. While previous studies have singularly explored a possible stress-CMV or a SES-CMV association, our investigation is the first to provide preliminary evidence that these predictors may interact. This finding adds to a small but growing literature demonstrating that SES can moderate the associations between stress and health. For example, in 5-year longitudinal study, Carroll et al. (2003) observed that cardiovascular reactivity to acute stress was a stronger predictor of blood pressure at follow-up among low-SES subjects than among high-SES subjects. Low SES has also been found to predict exaggerated stress reactivity of the immune (Brydon et al., 2004) and HPA-system (Gruenewald et al., 2006; Kunz-Ebrecht, Kirschbaum, & Steptoe, 2004). The exact mechanism responsible may involve exposure to more severe stressors (Grzywacz et al., 2004) or, alternatively, the same stressors having a larger impact due to impaired coping resources (Kristenson et al., 2004). The idea of impaired coping may translate to the biological level in the form of higher allostatic load (Seeman et al., 2010), which is consistent with the aberrant acute stress responses observed in low-SES individuals (McEwen & Seeman, 1999).

The current findings were not consistent with data demonstrating associations between lower SES and increased CMV-IgG levels in a nationally representative US sample (Dowd & Aiello, 2009). This may be due, in part, to the more restricted SES range of this occupational cohort. Specifically, in the cohort analyzed by Dowd and Aiello many had less than high school education while in the present study >95% had more than high school education. By

including the lowest SES groups, associations with CMV-IgG levels might involve factors linked to significant and prolonged deprivation, including distress (cf. Fiscella & Franks, 1997).

Inflammation has been shown to promote CMV reactivation *in vitro* (O'Connor & Murphy, 2012) and in experimental animal studies (Cook et al., 2006), but an association was not observed in the present study. This finding adds to a body of somewhat confusing evidence whereby some studies confirm an association between CMV and inflammation (e.g., Aiello et al., 2006; Fagundes et al., 2012; Roberts et al., 2010; Turner et al., 2014), but others do not (e.g., Bartlett et al., 2012; Bennett et al., 2012; Schmaltz et al., 2005).

Glucocorticoids have likewise been shown to cause CMV reactivation *in vitro* (Forbes et al., 1990; Tanaka, Ogura, Kamiya, Yoshie, et al., 1984). However, we did not observe an *in vivo* association between CMV-IgG and cortisol secretion, measured as the cortisol awakening response, diurnal cortisol AUC, or rate of cortisol decline. The lack of an association with diurnal HPA activity appears to contrast with the literature on EBV reactivation *in vivo* (Cacioppo et al., 2002; Glaser et al., 1994; Stowe et al., 2000). The reasons for this difference remain speculative at this point, but might reflect an intrinsic difference between the two viruses, which may explain the weak association between CMV and EBV antibody levels observed previously (Fagundes et al., 2012; Ling et al., 2003; Rahman et al., 1989). Also, considering the single day “snapshot” assessment of cortisol release in the present study, these findings should perhaps be followed up by more long-term assessments – for example via hair cortisol levels (cf. Stalder et al., 2013).

An important target for further research is also to determine the directionality between stress and viral reactivation. Naturalistic studies (e.g., academic stress, space flight) (Glaser et

al., 1999; Glaser et al., 1985; Mehta et al., 2000) as well as *in vitro* studies (Prösch et al., 2000) provided evidence that stress and stress hormones may drive viral replication. However, while less well-explored, there is also data to support a reverse causality, whereby CMV reactivation causes psychological changes. This neurological effect may occur due to CMV-induced inflammatory mediators not measured here (e.g., TNF- α , IL-6, IL-1, IFN- γ) that can act directly on the brain (Alcendor et al., 2012). Indirectly, CMV-induced inflammation may cause psychological changes by cytokine signaling, via afferent nerve fibers, and participate in pathways known to be involved in the development of anxiety, depression, and vital exhaustion (Goodkin & Appels, 1997; Raison et al., 2006; Silverman et al., 2007).

The present sample involved a disproportionate number of males (88%), which limits the generalizability of our findings to women. However, exploratory analyses did not provide an indication that associations were markedly different in women, and adjusting for gender also did not impact the results. Another potential limitation is that the sample was taken from a working population, and is subject to the ‘healthy worker’ phenomenon – the sample is enriched for those with a higher physical and psychological resilience more likely to stay in the work force (Sterling & Weinkam, 1985; Thygesen et al., 2011). Such selection may possibly result in an underestimation of the associations between distress and CMV reactivation due to a restriction of range (assuming that very high distress scores will be relatively absent). Finally, future studies may test if the observed associations would generalize to any of the other herpes viruses, such as EBV, HSV or VZV (Glaser & Kiecolt-Glaser, 2005).

In conclusion, in one of the largest studies to-date we observed remarkably consistent associations between CMV-specific IgG antibody levels, taken to signify viral reactivation,

and measures of psychological stress. These associations withstood adjustment for a range of confounding variables, including those that were predictive of CMV infection, and tended to be stronger in those with lower SES. Inflammation and HPA activity, as measured in the present study, did not appear to mediate these associations. Thus, stress-associated CMV reactivation seems to be a robust phenomenon that could be readily demonstrated in a healthy occupational sample, across a broad age range.

CHAPTER 4 : PERSONALITY AND CYTOMEGALOVIRUS

Abstract

Personality traits have been shown to predict health and longevity, but the exact underlying mechanisms are not clear. The pathway proposed here is that personality traits influence rates of infection by altering exposure and/or immune susceptibility to infectious agents. The current study used the herpes virus, cytomegalovirus (CMV), as an infection model to discern which of these aspects of immunity are impacted by personality traits, and as an immune-dominant infection linking personality to health.

In a large occupational cohort (N=887, mean age=44, 88% male), questionnaires assessed the Big 5 personality traits (extraversion, agreeableness, conscientiousness, neuroticism and openness), as well as demographic, socioeconomic (SES), and lifestyle factors. Plasma samples were analyzed for both the presence (to indicate infection status) and level (as a marker of reactivation) of CMV-specific IgG antibodies (CMV-IgG). Each personality trait was entered as an independent variable in logistic and linear regressions predicting CMV infection and CMV-IgG, respectively. The influences of the other traits were considered by mutually adjusting models for all other traits, and by clustering individuals exhibiting the profile known as resilience.

Every one-point increase in neuroticism was associated with 16% increased odds of CMV infection, OR(95% CI)=1.16 (1.05-1.29), $p<.01$, after adjustment for potential confounders and the other Big 5 traits. Linear regressions revealed that increased conscientiousness was associated with decreased CMV-IgG levels, $\beta=-.13$, $p=.05$, after the

same adjustments. None of the other Big 5 traits, or resilience classification, were associated with CMV infection status or CMV-IgG levels in mutually adjusted models.

This study shows for the first time that personality traits are associated with CMV infection and reactivation in a large sample of adults. The personality traits associated with CMV infection did not relate to CMV-IgG levels, and *vice versa*. This finding suggests that neuroticism may be related to increased exposure, while conscientiousness may have a protective effect on susceptibility to viral reactivation. Thus, this study provides preliminary evidence for CMV infection and its reactivation as a link between personality traits, health and longevity.

Introduction

There is an extensive literature linking personality with physical health and longevity in humans (Chapman et al., 2011; Smith & Mackenzie, 2006; Widiger & Seidlitz, 2002). For example, people who are high in some personality traits, such as emotional stability, general activity (a facet of extraversion) and conscientiousness, have been shown to live longer than those lower in these traits (Chapman, 2013; Chapman et al., 2010; Mroczek et al., 2009; Shipley et al., 2007; Terracciano et al., 2008). Similarly, clusters of personality traits have also been linked to health; individuals who have high resilience, characterized by low neuroticism and high scores on the other Big 5 personality traits (i.e., extraversion, agreeableness, conscientiousness and openness to new experiences) (Goldberg, 1990; McCrae & Costa, 1987), have been shown to have better subjective health, and reduced disease risk throughout the life course (Kinnunen et al., 2012; Tiainen et al., 2013; Wessman et al., 2012). Research is now exploring the potential mechanisms through which personality could influence health outcomes (reviewed in Smith & Mackenzie, 2006).

One proposed model is that personality may affect rates of infection, and therefore influence health and longevity (Segerstrom, 2000). For this to be a plausible mechanism, personality would have to be linked to the risk of infectious exposure (i.e. the likelihood of coming into contact with an infectious agent), and/or to the degree of vulnerability to that agent (i.e. the likelihood of becoming infected once exposed). There is evidence to support both contentions, although they have not yet been explicitly compared in a large sample of adults.

Personality consistently influences the types of behaviors that we engage in, and therefore is likely to influence our exposure to infectious diseases. For example, personality

influences social interaction, sexual behaviors and hand washing practices (Asendorpf & Wilpers, 1998; Hoyle et al., 2000), which are all linked to exposure rates for common infections (Aiello et al., 2008; Bates et al., 2004; Hawley et al., 2011). Further, a longitudinal study demonstrated that those with high neuroticism and low conscientiousness have a greater disease burden, as measured by the Charlson Comorbidity Index (CCI) (Sutin et al., 2013). The CCI is a weighted sum of 19 clinical conditions found to increase mortality risk, such as diabetes, congestive heart failure, and dementia (Charlson et al., 1987). Specifically, increases in a sub-trait of neuroticism were associated with a 26% increased risk of developing disease and a 36% risk of getting sicker at subsequent follow-ups (Sutin et al., 2013). Similar findings with behaviors related to extraversion have been found with parasite load in animal models (Koprivnikar et al., 2012).

As exposure is necessary, but not sufficient for infection to occur, the influence of personality on susceptibility to infection may also determine health and longevity. Personality-related changes in immune and neuroendocrine systems may increase vulnerability to infection in certain individuals (Miller et al., 1999; Sutin et al., 2012). For example, high neuroticism, and low extraversion and conscientiousness have been linked to markers of immune function, such as reduced vaccination efficacy, stimulated cytokine production, and natural killer cell cytotoxicity, as well as increased circulating markers of inflammation (reviewed in Cohen et al., 2012). These outcomes may stem from different personality types' propensity to feel and cope in a particular way, which in turn, corresponds to physiological responses to environmental stressors (Segerstrom, 2000). Indeed, personality traits (e.g., neuroticism) can contribute to a chronic and/or exaggerated activation of the stress response (Zobel et al., 2004), ultimately leading to long-term immunosuppression (Glaser &

Kiecolt-Glaser, 2005). On the other hand, increased resilience has been associated with reduced stress and a favorable profile of stress-related biomarkers (Petros et al., 2013). Taken together, personality may influence both exposure to infectious agents as well as the susceptibility that allows for infection and subsequent disease progression.

An elegant *in vivo* paradigm to study exposure and susceptibility simultaneously is measuring the distribution of, and immune responses to, the latent herpes virus, cytomegalovirus (CMV). CMV establishes life-long and mostly asymptomatic infection that is prevalent in 30%-90% of the population in developed countries. Prevalence increases nearly linearly with advancing age (Crough & Khanna, 2009; Staras et al., 2006) and correlates negatively with socioeconomic status (SES) (Dowd et al., 2009; Enders et al., 2012; Simanek et al., 2009). Temporary weakening of the immune system (e.g., due to psychological stress) triggers CMV to reactivate from its dormant state, termed latency, resulting in increased levels of CMV-specific IgG antibody (CMV-IgG) (Kuo et al., 2008; Van Zanten et al., 1995). Thus, a particularly attractive feature of CMV, as a model of infection, is that higher CMV-IgG levels represent a progressive failing of cellular immunity and poorer control over the virus (Glaser & Kiecolt-Glaser, 2005). Accordingly, CMV infection reflects a successful combination of both exposure and susceptibility, while elevated CMV-IgG antibody levels among those infected may specifically reflect impaired immunity and increased susceptibility. If a trait is associated with CMV infection and reactivation, then an effect on susceptibility is more likely; however, if it is associated with infection only, then exposure is a more plausible explanation. Comparing the effects of personality traits on these features of CMV may further elucidate their contribution to infectious exposure, susceptibility or both.

In recent years it has become evident that CMV infection also has clinical relevance. Congenital infection can cause serious neuronal damage to the developing fetus and accounts for more cases of birth defects and mental retardation per year than the better-known spina bifida, Down syndrome, or fetal alcohol syndrome (Cannon & Davis, 2005; Cheeran et al., 2009). Although asymptomatic in adults, CMV is also implicated in accelerating the biological aging of the immune system (Derhovanessian et al., 2009; Pawelec et al., 2009). In immune-compromised individuals (e.g., clinical illness, immunosuppressive therapy), CMV is responsible for a plethora of adverse outcomes (Söderberg-Nauclér, 2006; Varani & Landini, 2011), including increased mortality rates, especially among older adults (Gkrania-Klotsas et al., 2012; Pawelec et al., 2012; Simanek et al., 2011; Strandberg et al., 2009). Thus, any personality trait-related gradients for CMV infection or reactivation may have wider public health implications.

In a large sample of working-aged individuals, this study examined the relationship of the Big 5 personality traits (Goldberg, 1990; McCrae & Costa, 1987) and a resilience profile with CMV infection prevalence (exposure), and with plasma CMV-IgG concentration, which reflects impaired control over the virus among those infected (susceptibility). These analyses took into account potential confounders of these relationships, including socio-demographic (age, gender, marital status, SES) and lifestyle (smoking, alcohol intake, body mass index, physical activity) factors known to influence these relationships.

Materials and Methods

Participants

The present study was conducted on a subset of participants from the MIPH Industrial Cohort Studies (MICS) (N=887; 88% male; mean age 44; range 19-71) who took part in a

voluntary company health check (Li et al., 2013). The majority of subjects worked as non-managerial skilled workers, had at least completed an apprenticeship, and on average, had a BMI near the upper limit of the normal range. Participant characteristics are presented in Table 4.1. Participants received a personalized comprehensive health report. All data was anonymized before analysis, and this study was approved by the Ethical Committee of the Mannheim Medical Faculty, Heidelberg University. All participants signed informed consent.

Procedures

Participants arrived in the morning between 06:45h and 08:45h for their health check. In a quiet room away from their usual workplace, participants were seated separately to fill out questionnaires assessing demographics, medical history, and health behaviors, after a fasting blood draw. Anthropometric (e.g., height, weight, waist and hip circumference) and blood pressure measurements were carried out by trained study personnel.

Demographic and lifestyle data

Demographic data, including age, gender, and marital status, along with SES indicators (current job status, education level, or net monthly income in Euros), and lifestyle factors (e.g., smoking, alcohol consumption, activity level) were obtained by questionnaires used and validated in the MONICA study (Jönsson et al., 1999). Participants were identified as current smokers by self report (item: “Do you currently smoke?”). Smoking frequency was assessed as the number of cigarettes per day. Alcohol consumption was assessed as the cumulative number of alcoholic beverages (specified in equivalent units: e.g., bottle or can of beer 0.5 L/unit; red or white wine 0.2 L/unit; liquor 50 mL/glass) taken on average (response categories: not at all, 1-3 per month, 1 per week, 2-4 per week, 5-6 per week, 1 per day, 2-3

per day, 4-5 per day, 6 or more per day). Answers were converted into total average consumption in mean grams/week. Physical activity was derived from the self-reported average hours per week spent participating in various activities (e.g., walking, jogging, playing tennis). These activity durations were multiplied by their respective caloric expenditure, and averaged over seven days to yield a weighted sum of kilocalories per day.

Big 5 personality traits and the resilience profile

The personality traits extraversion, agreeableness, conscientiousness, neuroticism, and openness to new experiences were assessed with the short version of the Big Five Personality Inventory (BFI-10). This questionnaire comprises of 10 statements rated on a 5-point Likert scale (1 = “strongly disagree” to 5 = “agree strongly”). Summing the 2 items for each of the 5 traits results in the possible score range of 2-10, with higher scores indicating greater expression of the trait. The BFI-10 was developed simultaneously in several samples in both English and German, and compared to the original 44-item instrument (BFI-44) (Goldberg, 1992) and the NEO Personality Inventory Revised (NEO-PI-R) (Costa & McCrae, 1992). It has been shown to have significant levels of reliability and validity (Rammstedt & John, 2007). To define the resilience personality profile, we applied Ward’s hierarchical clustering procedure (Ward, 1963). This yielded two personality trait clusters that were significantly different on each of the Big 5 traits. The cluster that was low in neuroticism and high on all other traits were classified as resilient (Kinnunen et al., 2012; Tiainen et al., 2013).

Cytomegalovirus (CMV) antibody determination

CMV IgG antibody determination was carried out as described in Chapter 3 above (see pg. 38).

Statistical analysis

First, *t*-tests and χ^2 analyses were used to test for differences in Big 5 traits and covariates of interest between CMV+ and CMV– groups. Next, binary logistic regressions were utilized to calculate odds ratios (OR) and 95% confidence intervals (CI) for CMV infection from the Big 5 personality traits, adjusting for potential confounders, such as age, gender, marital status, lifestyle factors (smoking, alcohol, physical activity, and body mass index), and SES (job status and education), as well as the other Big 5 traits. Then, associations between the Big 5 personality traits and CMV-IgG levels among CMV+ individuals were assessed by linear regression, adjusting for the abovementioned potential confounders. Lastly, associations of the resilience profile (yes/no) with CMV infection and levels of CMV-IgG were tested with binary logistic regression and ANCOVA, respectively. Analyses were carried out with SPSS version 20 (IBM-SPSS, Chicago, IL, USA).

Results

Three hundred and twenty-nine participants (37%) were CMV+. Compared to their CMV– counterparts, CMV+ individuals were more likely to be older, married, current smokers, and to have lower education and job status (Table 4.1). Other demographic and lifestyle factors measured (i.e., gender, alcohol intake, BMI, waist-to-hip ratio, activity level, and income) did not differ by CMV status.

Table 4.2 shows the odds ratios of CMV infection for the Big 5 traits considered individually. Every one-point increment in agreeableness and neuroticism increased infection odds by 11% and 12%, respectively, after adjustment for age and gender. These associations were unaffected by further adjustment for marital status, SES (education and job status), and

lifestyle factors (smoking, activity level, alcohol intake, and BMI). Mutual adjustment for other personality traits revealed that neuroticism was significantly associated with a 16% increased odds of CMV infection after full adjustment (Model 4). None of the other Big 5 traits were significant predictors of CMV infection in the mutually adjusted model (all $p > .05$; Table 4.2).

Among those who were CMV+, none of the individual Big 5 traits were significantly associated with plasma CMV-IgG concentration in linear regressions (all $p > .05$). However, when the personality traits were entered together, higher conscientiousness was significantly associated with lower CMV-IgG, $\beta = -.13$, $p = .03$, after adjustment for age, gender, marital status, and SES (education and job status). This association remained significant after further adjustment for lifestyle factors (smoking, activity level, alcohol intake, and BMI), $\beta = -.13$, $p = .05$.

The cluster analysis revealed two personality groups: resilient ($n=336$) and non-resilient ($n=473$) individuals (missing=78). Logistic regressions revealed that being classified as resilient was unrelated to CMV infection odds in unadjusted or adjusted models (all $p > .05$). Likewise, CMV-IgG levels among CMV-infected individuals did not differ by resilience classification in any model (all $p > .10$).

Table 4.1: Participant characteristics.

	Overall (N=887)	CMV Status		p
		CMV Positive N=329 (37%)	CMV Negative N=558 (63 %)	
Age (years)	44.7 ± 10.1	46.6 ± 9.8	43.6 ± 10.1	<.001
Gender (% male)	88	86	89	.240
Current smoker (% yes)	26	32	23	.003
Cigarettes/day (in smokers)	13 ± 12	14 ± 11	13 ± 12	.729
Alcohol consumption (g/day)	19.7 ± 27.9	20.3 ± 26.2	19.3 ± 28.8	.592
Body mass index (kg/m ²)	24.5 ± 3.5	24.5 ± 3.6	24.6 ± 3.5	.657
Waist-to-hip ratio	0.92 ± 0.07	0.92 ± 0.07	0.92 ± 0.07	.621
Activity (kcal/day)	475.4 ± 478.9	467.3 ± 442.1	480.2 ± 499.7	.703
Married (%)	70	76	66	.003
Education (%)				.001
<i>College/University</i>	15	12	17	--
<i>Vocational training</i>	16	18	15	--
<i>Completed apprenticeship</i>	66	65	67	--
<i>No higher education</i>	3	5	1	--
Job status (%)				.009
<i>Division/Dept manager</i>	4	4	5	--
<i>Project Leader/process mgr</i>	19	17	20	--
<i>Skilled worker (non-mgr)</i>	71	70	72	--
<i>Semi-skilled worker</i>	6	9	4	--
Income (%)				.979
<i>> 4000 €/mo</i>	10	9	10	--
<i>2500 - 4000 €/mo</i>	40	41	40	--
<i>1500 - 2500 €/mo</i>	44	44	44	--
<i>< 1500 €/mo</i>	7	6	7	--

All are unadjusted comparisons of participant characteristics. A *t*-test was performed on continuous variables and a χ^2 for categorical variables. Values are mean ± SD unless otherwise stated.

Table 4.2: CMV infection odds ratios and 95% confidence intervals for the Big 5 personality traits.

	Model 1	Model 2	Model 3	Model 4
Extraversion	1.06 (0.97-1.15)	1.06 (0.97-1.16)	1.06 (0.97-1.16)	1.08 (0.99-1.19)
Agreeableness	1.11 (1.00-1.24)*	1.12 (1.00-1.24)*	1.12 (1.00-1.25)*	1.12 (1.00-1.25)†
Conscientiousness	1.02 (0.91-1.14)	1.00 (0.89-1.12)	1.01 (0.90-1.14)	1.00 (0.89-1.14)
Neuroticism	1.12 (1.02-1.23)*	1.10 (1.00-1.22)*	1.12 (1.02-1.24)*	1.16 (1.05-1.29)**
Openness	1.08 (0.98-1.19)	1.09 (0.99-1.20)	1.08 (0.98-1.20)	1.08 (0.97-1.19)

Model 1: adjusted for age and gender.

Model 2: Model 1 with additional adjustment for marital status, job status, & education.

Model 3: Model 2 further adjusted for smoking, alcohol intake, BMI and physical activity. Each trait was modeled in a separate regression.

Model 4: Model 3 with additional adjustment for the other Big 5 traits.

Values are odds ratios (95% confidence intervals). Significant at the * $p < .05$ level; † $p < .06$.

Discussion

This is the first study to investigate the association between the Big 5 personality traits and infection with, and reactivation of (measured as CMV-IgG level), CMV. Agreeableness and neuroticism were independently associated with an increased risk of CMV infection; however, only neuroticism remained significant when controlling for the other personality traits. This association was still significant after controlling for a range of potential confounders. However, none of the individual Big 5 personality traits were significantly associated with levels of CMV-IgG antibodies among those infected. When all personality traits were entered into the analysis together, conscientiousness was inversely associated with CMV-IgG levels, and remained significant after full confounder adjustment. These findings suggest that neuroticism is associated with increased exposure, while conscientiousness may contribute to reduced susceptibility to infection. These associations were not explained by socio-demographic or lifestyle factors; however, mutual adjustment for the other Big 5 traits influenced the appearance of associations, suggesting that the effects of one trait on immunity may be dependent on the configuration of the other traits.

The current associations of higher neuroticism and conscientiousness with increased CMV infection odds and reduced CMV-IgG levels, respectively, draws mixed support from another study relating CMV and temperament characteristics. Using Cloninger's Temperament and Character Inventory (TCI), Novotná et al. (2005) found, in a sample of N=533 military conscripts, that CMV infection was associated with lower scores for the trait novelty-seeking. TCI novelty-seeking is described as a tendency to respond with intense excitement to novel stimuli, thereby activating behavior (Wessman et al., 2012). Although the Big 5 does not measure novelty seeking, it could be considered the opposite of neuroticism which is characterized by anxiety, angry hostility, depression, self-consciousness, and vulnerability to stress (Costa & McCrae, 1992). Thus, the effects with CMV infection and higher neuroticism and lower novelty-seeking could be two sides of the same coin. However, the above study by Novotná and colleagues also found that novelty-seeking was inversely associated with continuous CMV-IgG levels (Novotná et al., 2005), but no relationship was found with neuroticism in our study.

Studies using other infection models (i.e., natural versus experimental exposure) have also found associations between neuroticism and infection. An earlier study, by Vickers and Hervig (1987) found, in multiple samples that neuroticism, and the sub-traits anxiety and depression, were associated with increased upper respiratory infection (URI) in two large groups (N=552 and N=591) of US Navy recruits over up to 53 days of basic training – a natural exposure context. Conversely, experimental exposure studies found no such associations between neuroticism, measured as negative emotional style or trait negative affectivity, and experimental susceptibility to the common cold (Cohen et al., 2012). The divergent results among these study designs could possibly provide insight into the

interpretation of the current findings. The laboratory exposure and quarantine phase of the experimental studies, in effect, removes social and environmental influences that are present in a natural context (Segerstrom, 2000). Thus, one could argue that the association with neuroticism found here may be attributed to its specific contribution to this exposure variance in the natural setting.

The observed inverse relationship between conscientiousness and CMV-IgG levels in the present study adds to a growing literature demonstrating protective effects of this trait on mental and physical well-being (Bogg & Roberts, 2013), as well as decreased mortality rates (Hagger-Johnson et al., 2012). It is possible that conscientiousness may exert its protective effects on health, in part, through its ability to buffer the effects of stress (Bartley & Roesch, 2011; Gartland et al., 2012; Murphy et al., 2013), possibly limiting the reactivation of CMV in those infected. When drawing from other infection models, the current findings with CMV reactivation (as a measure of susceptibility) contrast with one experimental exposure study that did not find an association between conscientiousness and cold development after experimental exposure (Cohen et al., 1997). However, it is worth mention that this study did not adjust for the other Big 5 traits, which may have confounded the association with their sickness measure, as has been demonstrated here. Taken together, the association of neuroticism with CMV infection and conscientiousness with CMV-IgG levels, but not vice versa, suggests that the personality factors related to infection prevalence do not continue to relate to control over the virus once infected. However, a combination of both exposure and susceptibility factors cannot be definitively ruled out.

The assumed direction of causation so far has been that personality traits result in increased infection odds, rather than infection influencing personality. This is supported by

longitudinal data, in which only modest changes in personality have been shown with increased disease burden (Sutin et al., 2013). Further, only 2% of the variance in novelty-seeking was explained by CMV infection in the study by Novotná et al. (2005). However, there is some evidence for reverse causation, whereby infectious exposures affect mood and behavior, which warrants consideration. For example, animal studies demonstrate that microbes in the gastrointestinal tract and viral infection can stimulate the central nervous system, modulate the stress response and produce changes in mood, anxiety and depression in the host (Ezenwa et al., 2012; Foster & Mcvey Neufeld, 2013; Silverman et al., 2007; Silverman et al., 2005). Similar behavioral effects have been documented in humans infected with *Toxoplasma gondii* (Flegr, 2007; Novotná et al., 2005). CMV infection could also lead to changes personality traits, via chronic low-grade inflammation and protracted sickness behavior (Almanzar et al., 2005; Dantzer et al., 2008; Khan et al., 2002; Maier & Watkins, 1998; Raison et al., 2006; Trzonkowski et al., 2003). This bi-directional aspect of the host-microbe relationship would benefit from longitudinal study. One model would be to follow CMV-negative transplant patients who are given a CMV+ organ, and track the time-course of any subsequent personality changes, compared to patients who received a CMV-compatible organ.

The current study is subject to some minor limitations. There were only two personality profiles distinguished by the cluster analyses using the short BFI-10, when at least five profiles have been identified in other research (Kinnunen et al., 2012). The current study simply classified the second group as ‘non-resilient’, but other profiles may exist within this group that differ in health behaviors and stress reactivity, and possibly, in CMV infection prevalence and CMV-IgG levels. The variance in this non-resilient group may have

contributed to the lack of associations found with resilience. In future studies, the use of a full length Big 5 inventory, with a wider range of scores, may allow for the creation of more discrete personality profiles to compare. The disproportionate number of males (88%) in the current study could have influenced the associations between individual traits, profiles and CMV. Documented gender differences exist in both health behaviors and personality profiles (Kinnunen et al., 2012; Tiainen et al., 2013) and females were found to have a higher CMV prevalence in US representative populations (Bate et al., 2010; Staras et al., 2006). However, there were no gender differences in CMV status in the current sample, and gender was statistically controlled throughout.

In conclusion, this study found a robust positive association between neuroticism and infection with the immune-dominant CMV and a negative association between conscientiousness and CMV-IgG levels among those infected in a large sample. Overall, these findings suggest that distinct personality traits are related to CMV exposure compared to that of susceptibility. The possibility of a bi-directional relationship between these factors cannot be discounted. The current results highlight infection history as a potential pathway between personality and health, and warrant further investigations into the mechanisms linking personality traits, particularly neuroticism and conscientiousness, and CMV.

CHAPTER 5 : ASSOCIATIONS BETWEEN PHYSICAL ACTIVITY FREQUENCY AND HIGHLY- DIFFERENTIATED T-CELLS

Abstract

Regular physical activity has been postulated to improve health and longevity, in part, via its ability to delay or reverse features of immune aging. A hallmark of immunosenescence is the accumulation of highly-differentiated T-cells in peripheral blood, which is partly driven by infection with cytomegalovirus (CMV). Preliminary studies have associated increased exercise and fitness with reductions in these cells; however, it is unclear whether these immune benefits are due to more habitual physical activity participation.

Participants in a European occupational cohort study, (N=1103, mean age=40, 88% male) reported the frequency of mild, moderate, and vigorous leisure-time physical activity, as well as socio-demographic, socioeconomic, and lifestyle factors by questionnaire. The numbers and proportions of circulating CD8⁺ effector-memory (EM; CD27⁻CD45RA⁻) and CD45RA⁺ effector-memory (EMRA; CD27⁻CD45RA⁺) T-cells, systemic inflammation and CMV infection status were assessed in whole blood and plasma.

More frequent mild physical activity was associated with lower numbers and proportions of EM and EMRA T-cells. Similarly, increased moderate physical activity frequency was inversely associated with EMRA T-cells. These associations remained significant after adjustment for a range of potential confounders, but were significantly attenuated by additional adjustment for CMV status in CD8⁺ T-cells. In age-stratified

comparisons, increasing physical activity frequency was associated with lower EM and EMRA T-cells in those in the upper age tertiles (aged >33 years).

This study is the first to provide evidence for an inverse relationship between leisure-time physical activity frequency and the accumulation of differentiated effector T-cells, with older individuals demonstrating the greatest benefits. These findings also highlight infection with CMV as a moderator of these effects in an intensity- and subset-dependent manner. Overall, increased frequency of physical activity may delay features of immunosenescence, which may be a mechanism linking physical activity with health and longevity.

Introduction

The health benefits of regular physical exercise are well-established (Autenrieth et al., 2011; Gillum & Obisesan, 2010; Richard et al., 2014; Woodcock et al., 2011), and are especially evident in older adults: compared to their less active or sedentary counterparts, older individuals who participate in habitual exercise have lower rates of and better prognosis for a range of diseases including cardiovascular disease, cancer and autoimmune diseases (Autenrieth et al., 2011; Plasqui, 2008; Soares-Miranda et al., 2014; Sofi et al., 2008; Tardon et al., 2005). Physically active individuals also have reduced risk of inflammatory diseases, due to the anti-inflammatory effects of exercise (Pedersen, 2009). These age-related causes of morbidity and mortality involve common immunological pathways, and it has therefore been proposed that exercise benefits health, in part, by enhancing immune function (Bigley et al., 2013; Romeo et al., 2010). A related, but more specific hypothesis is that exercise enhances health by delaying the age-associated decline in immune competence, known as immunosenescence (Drela et al., 2004; Kohut & Senchina, 2004; Pascoe et al., 2014; Senchina & Kohut, 2007; Simpson et al., 2012; Walsh et al., 2011).

A hallmark of immunosenescence is the accumulation of highly-differentiated effector memory (EM; CD27⁻CD45RA⁻) and CD45RA⁺ effector memory (EMRA; CD27⁻CD45RA⁺) cytotoxic (CD8⁺) T-cells in the peripheral blood (Fülöp et al., 2013; Spaulding et al., 1999). The latent herpes virus, cytomegalovirus (CMV), is known to potently accelerate the accumulation of these T-cells, whereby those infected (CMV⁺) have on average 3- to 4-fold higher circulating numbers than in those uninfected (CMV⁻) (Chidrawar et al., 2009; Derhovanessian et al., 2011; Fülöp et al., 2013; Koch et al., 2007; Kuijpers et al., 2003; Pawelec et al., 2006; Pita-Lopez et al., 2009; Turner et al., 2014; Wallace et al., 2011;

Wertheimer et al., 2014). These EM and EMRA T-cells are characterized by a diminished proliferative capacity, potent pro-inflammatory cytokine production and short telomere lengths (Macaulay et al., 2013). Correspondingly, the accumulation of these cells has been mechanistically linked to key features of immune aging, such as poorer vaccination efficacy, impaired immune responses to previously encountered pathogens, elevated systemic inflammation, and decreased average leukocyte telomere length (Effros, 2007; Goronzy & Weyand, 2013; Messaoudi et al., 2004). Therefore, factors which combat the accumulation of these differentiated T-cells may arguably alleviate the deleterious effects of immunosenescence.

Importantly, studies of aerobic fitness and exercise training have provided preliminary evidence that habitual exercise is associated with lower proportions and frequencies of highly-differentiated T-cells in circulation (Spielmann et al., 2011; Woods et al., 2003). For example, Spielmann et al. (2011) found that those in the highest tertile of aerobic fitness, measured as maximum oxygen uptake (VO_{2max}), had 37% lower proportions of highly-differentiated CD8+ T-cells compared to those in the lowest tertile. On the other hand, there is also evidence that the prolonged, exceptionally intense training regimens, as undertaken by elite athletes, may instead promote an aged immune profile and increased infection risk (Gleeson & Walsh, 2012; Moro-Garcia et al., 2014; Prieto-Hinojosa et al., 2014). These findings imply that the observed benefits of improved fitness on features of immunosenescence may lie in participation in more frequent, but not necessarily more intense physical activity (Simpson & Bosch, 2014). However, few studies are available linking habitual physical activity with T-cell differentiation. Evidence for such an association can be inferred from studies showing longer leukocyte telomere length among those reporting higher levels of leisure-time physical

activity, compared to those least active (Cherkas et al., 2008; Ludlow et al., 2008). Thus, frequent participation in physical activity may help buffer the effects of age and CMV infection on the accumulation of highly-differentiated T-cells, and studies linking these factors in larger samples, preferably stratified by intensity, are warranted.

To address these issues, the present study utilized a large cohort to examine the relationship between the frequency of leisure-time physical activity and the numbers and proportions of highly-differentiated T-cell subsets. Analyses were adjusted for CMV infection status, and potential confounders and mediators of these relationships were also taken into account, such as age, socioeconomic status, lifestyle factors (smoking, alcohol intake, BMI), and systemic inflammation (Simanek et al., 2011). Particularly, age has been shown to interact with leisure-time physical activity to predict mortality, whereby the greatest reduction in mortality risk was observed among physically active individuals aged 60 years and over compared to their inactive counterparts (Richard et al., 2014). Thus, further analyses examined the impact of physical activity frequency on immunosenescence within age groups. It was hypothesized that increased physical activity would be associated with decreased numbers and proportions of highly-differentiated CD8⁺ T cells and it was anticipated that this effect would be more pronounced in older individuals. In light of evidence that a specialized population of T-cells bearing a non-conventional gamma-delta ($\gamma\delta$) T-cell receptor ($\gamma\delta$ TCR⁺ T-cells) also expand considerably in a CMV-specific manner (Alejane et al., 2014; Dechanet et al., 1999; Pitard et al., 2008; Vermijlen et al., 2010), the above analyses were repeated for this cell subset.

Materials and Methods

Participants

The present study was conducted among employees (N=1103; 88% male; mean age 40 \pm 11; range 18-64) of a large airplane manufacturer in the south of Germany who took part in a voluntary health check offered by the company. Participant characteristics are presented in Table 5.1. Participants received a personalized comprehensive health report. All data was anonymized before analysis, and this study was approved by the ethical committee of the Medical Faculty Mannheim, Heidelberg University. All participants signed informed consent.

Procedures

Participants arrived at a location away from their usual workplace in the morning between 06:45h and 08:45h for their health check. After a fasting venous blood draw and medical examinations, participants were seated in a quiet room to fill out questionnaires measuring demographic, medical, and health behavior data. Anthropometric (e.g., height, weight, waist and hip circumference), as well as blood pressure and resting heart rate measurements were carried out by trained study personnel.

Flow cytometry

T-cell phenotypes were assessed by flow cytometry. Whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) coated tubes (Sarstedt, Nümbrecht, Germany), stored at room temperature, and prepared within 1 hour of collection. Briefly, 30 μ L of whole blood was stained with a combination of the following monoclonal antibodies: CD3 APC-Cy7, CD4 PerCP, $\gamma\delta$ TCR PE, CD8 APC, (BD Biosciences, San José, CA), CD45RA FITC, and CD27 PE-Cy7 (BD Pharmingen, San Diego, CA). Following 20 minutes

of incubation at room temperature in the dark, 1.5 mL of BD FACS lysing solution was added to the mixture, and incubated for another 15 minutes. After a 7-minute centrifugation at 700xg, the supernatant was removed; the pellet was re-suspended in 250 µL of 2% paraformaldehyde solution until analyzed. Data was collected using a FACSCanto II flow cytometer (BD Biosciences, San José, CA) and the FACS Diva software (BD Biosciences, San José, CA). Spectral overlap was electronically compensated for using single labelled antibody tubes. Following acquisition, FCS files were transferred to a third party software program (FlowJo v7.6.5, Tree Star, Inc., Ashland, OR) for analysis.

Cytomegalovirus (CMV) and biochemical analyses

Plasma from fasted blood samples were obtained and stored in small aliquots at -80C until analysis. Evidence of previous CMV infection (serostatus) was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (BioCheck, Inc., CA, USA) according to manufacturer instructions. Participants with a borderline seropositive result, representing a calculated index score >0.85 and <1.15 , were retested (N=9). If they remained borderline, only subjects with index scores above 1.00 were considered positive (CMV+), per manufacturer instructions. The sensitivity, specificity, and accuracy of the test were reported as 95.0, 96.7 and 96.0%, respectively. High-sensitivity C-reactive protein (hs-CRP) was analyzed by immunonephelometry using a Behring Nephelometer II (High Sensitivity CRP, Dade Behring). The detection limit for hs-CRP was 0.015 mg/L, with intra-assay and inter-assay CV% $< 10\%$.

Physical activity frequency

Three items, based on the Godin Leisure-Time Physical Activity Questionnaire (Godin & Shephard, 1985, 1997), assessed the frequency of mild, moderate, and vigorous physical activity. Mild physical activity was defined as engaging activity, such as walking or light garden work that minimally accelerated their heartbeat for at least 20 minutes. Moderate physical activity (e.g., fast walking, riding a bicycle) accelerated their heartbeat, but didn't make them sweat, whereas vigorous physical activity really got them sweating. Participants were asked how often in the past 6 months they engaged in each level of physical activity. Response categories were: (1) hardly ever; (2) one to three times per month; (3) about once a week; (4) more than once a week; (5) three times or more often per week.

Demographic and lifestyle data

Demographic data, including age, gender, marital status, SES indicators (measured as current job status, manual occupation, or shift worker), and lifestyle factors (e.g., smoking, alcohol, hours per week in physical activity) were obtained by questionnaires used and validated in the MONICA study (Jönsson et al., 1999). Participants were defined as current smokers if they answered yes to the question: "Do you currently smoke?" These were treated as potential confounding factors, and were controlled in the analyses as described below.

Statistical Analysis

To discern differences between CMV+ and CMV– individuals, continuous and categorical variables were compared using t-tests and χ^2 analyses, respectively. The analyses for each of the three intensities of physical activity (mild, moderate, vigorous) were followed up by binary logistic regression to calculate odds ratios (OR) and 95% confidence intervals

(95% CI) for CMV infection. These analyses were adjusted for potential confounding factors that may impact physical activity participation, CMV infection and reactivation, including age, gender, marital status, lifestyle (smoking, alcohol, BMI, activity) and SES (job status and manual occupation) and systemic inflammation (hs-CRP).

The association of mild, moderate and vigorous physical activity frequency with the numbers and percentages of highly-differentiated CD8⁺ EM and EMRA T-cells was assessed by linear regression. These associations were likewise controlled for the above socio-demographic, lifestyle, SES factors and hs-CRP. Due to its strong association with the accumulation of these cells, a second model was added to the analyses, further adjusting for CMV status. These linear regression analyses were repeated using $\gamma\delta$ TCR⁺ T-cell subsets as dependent variables.

To examine the impact of age on these relationships, the participants were divided into three equal age groups, and the numbers of EM and EMRA CD8⁺ T-cells were compared by analysis of covariance (ANCOVA). Bonferroni-corrected post-hoc comparisons were used to examine the effects of the frequency of physical activity (<1x/wk, 1-2x/wk, and >2x/wk) on CD8⁺T-cell subset numbers within each age tertile. Analyses were performed with SPSS version 20 (IBM-SPSS, Chicago, IL, USA).

Results

Differences between CMV⁺ and CMV⁻ individuals

As shown in Table 5.1, 400 (36%) participants were CMV⁺. On average, CMV⁺ participants tended to be older and married/cohabitating. CMV⁺ individuals were also more likely to be female, current (or former) smokers, to drink alcohol less frequently, and to have lower socioeconomic status (low job status and manual occupations, and work in shifts).

Among smokers, the amount of cigarettes smoked per day did not differ according to CMV status (Table 5.1). Table 5.2 shows the unadjusted participant physical characteristics. CMV+ individuals were less likely to engage in mild ($p=.020$), moderate ($p=.041$), but not vigorous ($p=.335$) physical activity more than twice per week, compared to their CMV– counterparts. However, no other physical characteristics, including BMI, waist-to-hip ratio, self-reported hours of activity, and hs-CRP, differed by CMV status in unadjusted analyses (Table 5.2).

After full adjustment (age, gender, marital status, smoking, alcohol, BMI, job status, manual occupation and hs-CRP), binary logistic regressions revealed that, compared to inactive individuals, the odds of CMV infection were 54% and 43% lower among those participating in mild, OR (95% CI) = .46 (.26 - .82), $p=.008$, or moderate, OR (95% CI) = .57 (.35 - .92), $p=.021$, physical activity three or more times per week, respectively. The frequency of vigorous physical activity was not significantly associated with odds of CMV infection after full adjustment ($p=.49$).

Lower differentiated CD8+ T-cells with increased mild and moderate physical activity depends on CMV infection status

Table 5.3 shows the association between the frequency of physical activity and the number and proportion of T-cell subsets in all participants. Increased frequency of mild physical activity was associated with decreased numbers and proportions of CD8+ EM and EMRA T-cells after adjustment for age, gender, marital status, lifestyle factors (smoking and alcohol, BMI), and SES (job status and manual occupation) and hs-CRP. Moderate physical activity frequency was also inversely associated with numbers and percentages of CD8+ EMRA T-cells. In a second model, additionally adjusting for the effects of CMV status on T-cell accumulation, the above associations were reduced to non-significance ($p<.06$) (Table

5.3). Due to these attenuations, the possible interaction effect of CMV infection and physical activity frequency on CD8+ EM and EMRA T-cell numbers and percentages was tested by moderation analyses. However, these analyses did not reveal any evidence for moderation ($p > .07$ for all; data not shown).

Moderate and vigorous physical activity also showed a significant negative relationship with numbers and proportions of total CD8+ T-cells. Only the associations with vigorous physical activity remained significant after final adjustment for CMV. Vigorous physical activity was not associated with CD8+ EM and EMRA T-cell subsets (Table 5.3).

Age differences in differentiated CD8+ T-cells

Figure 5.1 shows the associations between physical activity frequency and numbers of CD8+ EM and EMRA T-cells within each age tertile. First, basic comparisons of T-cell numbers between age groups revealed a significant main effect of age for both T-cell subsets across all categories (mild, moderate, and vigorous) of physical activity. Specifically, T-cell subset numbers in individuals aged 34-45 yrs and 46-64 yrs were, in general, significantly higher compared to those 18-33 yrs. There were no differences observed between those 34-45 yrs and 46-64 yrs (Figure 5.1). Fully adjusted analyses revealed that the main effect of age remained significant for EM and EMRA T-cell subsets after controlling for gender, marital status, SES (job status and manual occupation), and lifestyle factors (smoking, alcohol, BMI, and activity), as well as additional adjustment for CMV status.

Age-dependent associations between physical activity frequency and the accumulation of differentiated CD8+ T-cells

The possibility of an age-dependent effect of physical activity on T-cell numbers was tested by post-hoc comparisons within each age tertile. Physical activity frequency was not

associated with CD8+ EM and EMRA numbers in those aged 18-33 yrs. Among individuals aged 34-45 yrs, CD8+ EM T-cells were significantly lower in those reporting mild physical activity 1-2 times per week, and moderate physical activity more than once per week. Within the same age group, CD8+ EMRA were significantly lower in those participating in mild physical activity more than twice per week. Among those aged 46-64 yrs, CD8+ EMRA T-cell numbers were lower in those reporting mild physical activity more than once per week (Figure 5.1). With the exception of lower numbers of CD8+ EMRA numbers in those aged >33 yrs reporting mild physical activity more than twice per week (both $p < .05$), the above associations did not survive full adjustment. Further adjustment for CMV status attenuated these remaining associations. Vigorous physical activity frequency was not associated with significantly lower EM or EMRA numbers in any age group.

Increased mild and moderate physical activity frequency is associated with lower differentiated $\gamma\delta$ TCR+ T-cells

The linear regression analyses above were repeated for $\gamma\delta$ TCR+ T-cell subsets, which are also known to expand considerably in response to CMV infection. As shown in Table 5.4, more regular mild and moderate physical activity was associated with lower numbers and percentages of $\gamma\delta$ TCR+ EMRA T-cells. Mild physical activity was also negatively associated with the numbers of $\gamma\delta$ TCR+ EM T-cells after full adjustment. In a second model, with additional adjustment for CMV status, the relationships between $\gamma\delta$ TCR+ subsets and moderate, but not mild physical activity remained significant (Table 5.4). Moderation analyses did not reveal any significant interaction terms between mild and moderate physical activity and $\gamma\delta$ TCR+ T-cell subsets (data not shown). Vigorous physical activity frequency was not significantly associated with any of the $\gamma\delta$ TCR+ T-cell subsets (Table 5.4).

Table 5.1: Participant socio-demographic characteristics

	Total (N=1103)	CMV Status		p
		Positive N=400(36%)	Negative N=703(64%)	
Age	40.1 ± 11.0	41.5 ± 11.1	39.3 ± 10.8	<.001
Gender (% male)	87.7	84.5	89.5	.019
Alcohol intake (%)	--	--	--	<.001
<i>0-2x/month</i>	24.5	30.2	21.3	
<i>1-2x/wk</i>	30.1	32.7	28.6	
<i>3-7x/wk</i>	45.4	37.1	50.1	
Smoking (%)	--	--	--	.027
<i>Never smoker</i>	46.7	41.2	49.8	
<i>Former smoker</i>	24.8	27.0	23.5	
<i>Smoker</i>	28.6	31.8	26.7	
Cigarettes per day (in smokers)	14 ± 8	15 ± 7	13 ± 8	.091
Married/Co-habiting (%)	77.4	81.3	75.2	.028
Job status (%)	--	--	--	<.001
<i>Division/dept mgr</i>	3.9	2.1	4.9	
<i>Project leader/process mgr</i>	15.1	13.5	16.0	
<i>Worker (managerial)</i>	6.3	5.5	6.7	
<i>Skilled worker (non-mgr)</i>	65.4	64.6	65.9	
<i>Semi-skilled worker</i>	9.3	14.2	6.4	
Shift worker (% yes)	28.1	34.0	24.7	.002
Manual occupation (% yes)	49.6	55.0	46.5	.008

All are unadjusted comparisons of participant characteristics. A *t*-test was performed on continuous variables and a χ^2 for categorical variables. Values are mean ± SD unless otherwise stated.

Table 5.2: Participant physical characteristics.

	Total N=1103	CMV Status		p
		Positive N=400(36%)	Negative N=703(64%)	
Mild PA ^a (%)	--	--	--	.020
<1x/wk	21.2	25.5	18.7	
1-2x/wk	53.5	52.3	54.2	
>2x/wk	25.3	22.1	27.1	
Moderate PA (%)	--	--	--	.041
<1x/wk	28.1	31.4	26.2	
1-2x/wk	50.2	50.6	50.0	
>2x/wk	21.7	17.9	23.8	
Vigorous PA (%)	--	--	--	.335
<1x/wk	31.0	33.2	29.7	
1-2x/wk	52.7	52.2	52.9	
>2x/wk	16.3	14.5	17.3	
Body mass index (kg/m ²)	24.5 ± 4.0	24.5 ± 4.0	24.5 ± 4.1	.927
Waist-to-hip ratio	0.90 ± .08	0.90 ± .08	0.90 ± .07	.200
Activity ^b (h/wk)	7.0 ± 7.5	7.1 ± 9.4	7.0 ± 6.2	.355
Resting HR ^c (bpm)	72 ± 12	72 ± 12	71 ± 12	.080
Systolic BP ^d (mmHg)	136 ± 14	135 ± 14	136 ± 13	.280
Diastolic BP (mmHg)	78 ± 12	78 ± 12	78 ± 12	.665
hsCRP ^e (mg/L)	0.17 ± 0.30	0.16 ± 0.23	0.18 ± 0.33	.517

All are unadjusted comparisons of participant characteristics. A *t*-test was performed on continuous variables and a χ^2 for categorical variables. ^aPhysical activity; ^bleisure-time physical activity ^cheart rate; ^dblood pressure; ^ehigh sensitivity c-reactive protein. Values are mean ± SD unless otherwise stated.

Table 5.3: Standardized regression coefficients for CD8+ T-cell subset numbers and percentages and reported frequency of mild, moderate, and vigorous physical activity.

		Mild		Moderate		Vigorous	
		Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
CD8+	#	-.062*	-.036	-.072*	-.054	-.076*	-.069*
	%	-.037	-.010	-.076*	-.058 [†]	-.077*	-.070*
CD8+ EM ^a	#	-.083**	-.057 [†]	-.025	-.007	-.018	-.011
	%	-.082**	-.057 [†]	-.030	-.013	-.013	-.007
CD8+ EMRA ^b	#	-.101***	-.051 [†]	-.073*	-.039	-.024	-.011
	%	-.093**	-.041	-.065*	-.030	-.013	.0002

Model 1: Adjusted for age, gender, marital status, lifestyle factors (smoking, alcohol, BMI), and SES (job status, manual occupation), and c-reactive protein (CRP).

Model 2: Model 1 further adjusted for CMV status.

Significant at the *p<.05, **p<.01, ***p<.001, and [†]p<.06 level. ^aEffector memory;

^bCD45RA+ effector memory.

Table 5.4: Standardized regression coefficients for $\gamma\delta$ TCR+ T-cell subset numbers and percentages and reported frequency of mild, moderate, and vigorous physical activity

		Mild		Moderate		Vigorous	
		Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
$\gamma\delta$ TCR+	#	-.056	-.041	-.050	-.040	-.036	-.032
	%	-.031	-.019	-.035	-.027	-.022	-.019
$\gamma\delta$ TCR+ EM ^a	#	-.065*	-.059	-.016	-.011	-.034	-.033
	%	-.047	-.049	.009	.007	-.019	-.019
$\gamma\delta$ TCR+ EMRA ^b	#	-.080*	-.043	-.087**	-.061*	-.038	-.029
	%	-.072*	-.032	-.084**	-.057*	-.018	-.008

Model 1: Adjusted for age, gender, marital status, lifestyle factors (smoking, alcohol, BMI), and SES (job status, manual occupation), and c-reactive protein (CRP).

Model 2: Model 1 further adjusted for CMV status.

Significant at the *p<.05 & **p<.01 level. ^aEffector memory; ^bCD45RA+ effector memory.

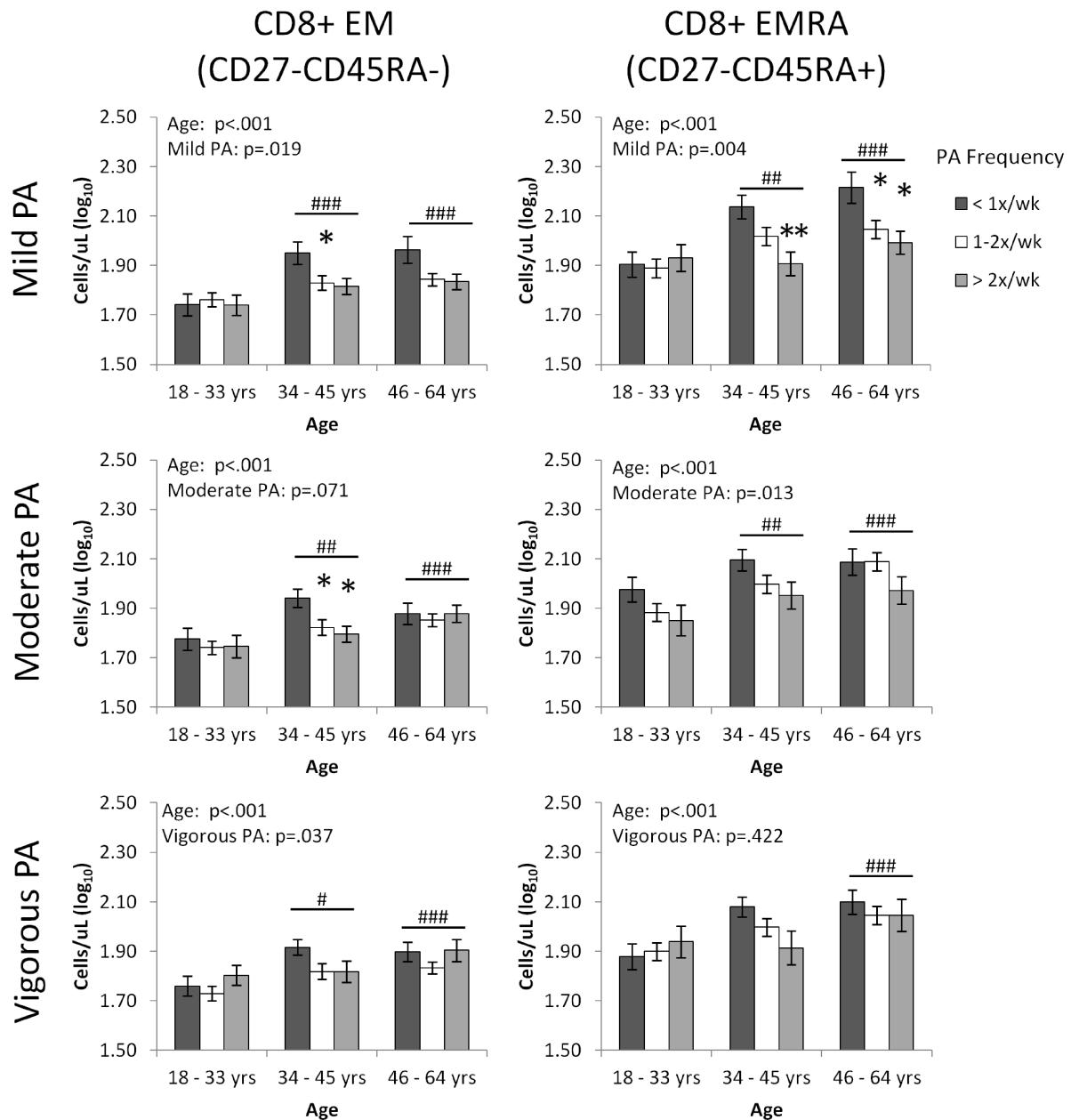


Figure 5.1: Unadjusted comparisons of numbers of CD27-CD45RA+/- T-cell subsets by age and physical activity frequency. Significantly different from $< 1x/wk$ group at the $*p < .05$ & $**p < .01$ level. Significantly different from 18-33 yrs group at the $\#p < .05$, $##p < .01$ & $###p < .001$ level. Values are Mean \pm SEM.

Discussion

The present study found that a higher frequency of both mild and moderate physical activity was associated with lower EMRA CD8⁺ and $\gamma\delta$ TCR⁺ T-cells, and mild physical activity was additionally associated with lower EM CD8⁺ T-cells. These findings withstood adjustment for a wide range of socio-demographic and lifestyle factors, as well as systemic inflammation (hs-CRP), indicating that physical activity patterns have a robust impact on the accumulation of highly-differentiated T-cells. Overall, these results provide evidence for the first time that older individuals participating in increased mild and moderate, but not vigorous physical activity, display lower numbers and proportions of highly-differentiated T-cells, indicative of a delayed onset of immunosenescence. For the first time, this study examined the specialized subset of $\gamma\delta$ TCR⁺ T-cells in the context of physical activity frequency and found a similar pattern of associations as CD8⁺ T-cells after full adjustment.

These findings also highlight infection history as a possible co-determinant of these relationships. Adjustment for CMV status influenced the observed relationships in a subset-dependent manner, whereby CMV attenuated the associations of mild and moderate physical activity with CD8⁺ T-cells, whereas with $\gamma\delta$ TCR⁺ T-cells, moderate physical activity associations were unaffected. A possible explanation for this pattern may lie in intrinsic differences between these two cell types. Although both CD8⁺ and $\gamma\delta$ TCR⁺ T-cells expand similarly in response to CMV infection and express a differentiated phenotype (Couzi et al., 2009; Pitard et al., 2008; Van De Berg et al., 2008), the CMV-specific $\gamma\delta$ TCR⁺ T-cell population require additional stimulation for activation (Alejenez et al., 2014). Thus, the differential attenuation by CMV may reflect distinct effects of a given physical activity intensity on the mobilization of CMV-specific CD8⁺ versus $\gamma\delta$ TCR⁺ EM and EMRA T-cells

(Simpson, 2011). These $\gamma\delta\text{TCR}^+$ T-cells have a greater exercise intensity-dependent mobilization than CD8^+ T-cells (Anane et al., 2009), and future studies, stratifying these responses by CMV status, would help confirm or refute this speculation.

In age-stratified comparisons, increasing physical activity frequency was associated with lower EM and EMRA T-cells in the upper two age tertiles (>33 years), but not in the lowest tertile (≤ 33 years). The null findings among the younger individuals may reflect less life-long pathogen exposure and a more diverse T-cell pool compared to their older counterparts (Le Saux et al., 2012; Palmer, 2013; Pawelec, 2012; Qi et al., 2014). Assuming that the effects of physical activity restore balance to dysregulated immune functioning, it would not be expected to alter the already competent immune system in these younger individuals. The associations in the upper two age tertiles may allude to the benefits of a more life-long proclivity to physical activity participation in these older adults. Studies utilizing exercise interventions up to 12 months provide little evidence of improvements in T-cell immunity, despite marked increases in fitness level. This may suggest that longer periods of participation in physical activity are needed to achieve the immune benefits observed in cross-sectional studies (Simpson et al., 2012). Assuming frequent exercise-related mobilization and subsequent deletion of these cells as a mechanism, engaging in habitual physical activity throughout the lifespan could culminate in frequent exercise-induced clearance of highly-differentiated T-cells and/or stimulating preservation of thymic output, possibly via frequent cell turnover (Simpson, 2011). Studies aimed at determining a potential optimal range of physical activity (in terms of frequency, intensity and volume) needed to positively alter the composition of the blood T-cell pool are warranted (Simpson & Bosch, 2014).

The current study has limitations. The cross-sectional study design does not allow for causal inferences between physical activity and the accumulation of highly-differentiated T-cells. The results are presented here in the orientation consistent with previous research reporting immune benefits, including longer leukocyte telomere length (Cherkas et al., 2008) and lower proportions of so-called senescent T-cells (Spielmann et al., 2011) with increased physical activity participation and aerobic fitness. However, progressive T-cell differentiation and low-grade inflammation have been suggested to occur simultaneously and reciprocally reinforce one another (Macaulay et al., 2013; Moro-Garcia et al., 2013). This pro-inflammatory state may feed into the so-called sickness behavior and an associated withdrawal from physical activity (Maier & Watkins, 1998). The associations found in the current study were independent of systemic inflammation, as measured by hs-CRP, suggesting other pathways. Another limitation is the lack of an objective fitness measure. However, the current study did measure resting heart rate. Resting heart rate is considered a reliable proxy of physical fitness, has concurrent validity with self-reported physical activity, and is predictive of mortality in epidemiological studies (Batty et al., 2010; Emaus et al., 2010; Jensen et al., 2012; Jensen et al., 2013). There were no differences in resting heart rate with CMV infection in the current study, and the current associations between physical activity frequency and differentiated T-cells was unaffected when resting heart rate was entered into an additional model.

In summary, this is first study to provide evidence for an inverse relationship between physical activity frequency and the accumulation highly-differentiated CD8⁺ and $\gamma\delta$ TCR⁺ T-cells. These findings generalize preliminary findings with exercise and fitness to the wider category of physical activity. These relationships were robust to a range of socio-

demographic, lifestyle, and inflammatory variables previously associated with physical activity participation and immunity. Attenuation by CMV status occurred in an intensity- and subset-dependent manner, suggesting that infection history may influence the requirements in physical activity frequency, intensity and volume necessary for beneficial effects on immune composition. Lower levels of highly-differentiated T-cells observed with more frequent physical activity was only apparent among those in the upper two age tertiles (34 years and older), which emphasizes that physical activity becomes particularly important as one ages, and may suggest that the manifestation of immune enhancements from habitual participation becomes apparent later in life. Overall, the current study suggests an alleviating effect of physical activity on immunosenescence, which may have considerable implications in the wide-spanning benefits on health and mortality.

CHAPTER 6 : ELEVATED HBA1C LEVELS AND THE ACCUMULATION OF DIFFERENTIATED T-CELLS IN CMV+ INDIVIDUALS

Abstract

Biological aging of the immune system, or immunosenescence, predicts poor health and increased mortality. A hallmark of an aging immune system is the accumulation of differentiated CD8⁺ T-cells (CD27⁻CD45RA^{+/-}; dCTL), which is partially driven by latent infection with cytomegalovirus (CMV). Immune impairments reminiscent of immunosenescence have also been observed in hyperglycemia, and *in vitro* studies have identified mechanisms by which elevated glucose levels can lead to the accumulation of dCTL. The present study explored associations between glucose dysregulation and markers of immunosenescence in CMV-positive and -negative individuals.

Participants in a European occupational cohort study (N=1103, mean age=40, 88% male) were assessed for HbA1c and fasting glucose levels, cardiovascular risk factors (e.g., lipid profile), numbers of circulating effector-memory (EM; CD27⁻CD45RA⁻) and CD45RA⁺ effector-memory (EMRA; CD27⁻CD45RA⁺) T-cells, and CMV infection status. Self-report and physical examination assessed socio-demographic, health risk (e.g., diabetic status, metabolic syndrome components), and lifestyle factors.

Among CMV+ individuals (n=400), elevated HbA1c was associated with increased numbers of EM (B=2.75, p<.01) and EMRA (B=2.90, p<.01) CTL, which was robust to adjustment for age, gender, socio-demographic variables, and lifestyle factors. Elevated EM

CTL was also positively associated total cholesterol ($B=.04$, $p<.05$), applying similar adjustments. No associations were observed in CMV– individuals.

The present study identified consistent associations between unfavorable glucose and lipid profiles and accumulation of dCTL in CMV+ individuals. These results provide evidence that the impact of metabolic risk factors on immunity and health can be co-determined by infectious factors, and provide a novel pathway linking metabolic risk factors with accelerated immunological aging.

Introduction

The progressive impairment of immunity with age, known as immunosenescence, is thought to underlie increased infection risk and mortality (Effros et al., 2005; Macaulay et al., 2013; Trzonkowski et al., 2003; Turner et al., 2014) and may also contribute to several other age-associated complications, including low-grade inflammation and increased cardiovascular disease (CVD) risk (Franceschi, Bonafè, et al., 2000; Freitas et al., 2012; High et al., 2005; Lindstrom & Robinson, 2010; Luz Correa et al., 2014; Montoya-Ortiz, 2013; Prelog, 2006; Salpea & Humphries, 2010; Sansoni et al., 2008). Infection with cytomegalovirus (CMV) has been shown to accelerate features of immunosenescence (Chidrawar et al., 2009; Dowd et al., 2013; Qiu et al., 2008; Turner et al., 2014; Van De Berg et al., 2010). This herpes virus establishes a life-long infection during which the virus remains latent, interrupted with periods of non-clinical reactivation. The resultant activation of CMV-specific T-cells leads to a marked accumulation of differentiated memory cytotoxic T-cells (dCTL; CD27⁻CD45RA^{+/-}), which can be subdivided into effector memory (EM; CD27⁻CD45RA⁻) and CD45RA⁺ effector memory (EMRA; CD27⁻CD45RA⁺) (Chidrawar et al., 2009; Van De Berg et al., 2008; Wallace et al., 2011). Indeed, infected (CMV+) individuals have on average 3- to 4-fold higher dCTL numbers compared to those uninfected (CMV-), although large individual differences exist (Derhovanessian et al., 2011; Koch et al., 2007; Kuijpers et al., 2003; Pawelec et al., 2006; Pita-Lopez et al., 2009; Wertheimer et al., 2014).

The accumulation of dCTLs may make a material contribution to the acceleration of immunosenescence (Messaoudi et al., 2004) and is thought to provide a mechanism through which immunosenescence may be associated with health outcomes, such as CVD (Macaulay et al., 2013). For example, these T-cells show a high production of pro-inflammatory

cytokines, have short telomeres, and have an aberrant proliferative capacity (Akbar & Henson, 2011; Almanzar et al., 2005; Hamann et al., 1997; Moro-Garcia et al., 2012; Wallace et al., 2011). It is through the accumulation of dCTL that CMV may be associated with hallmarks of an aged immune system.

Significantly, many of the immune system impairments that have been associated with aging resemble those of chronic hyperglycemia. For example, impaired glucose tolerance and diabetes are associated with poor control of infection (Allard et al., 2010; Joshi et al., 1999; Shah & Hux, 2003), impaired vaccination responses (Egawa et al., 2014), elevated inflammatory activity (Esposito et al., 2002; Marfella et al., 2003), and shorter leukocyte telomere length (Adaikalakoteswari et al., 2007; Salpea & Humphries, 2010). These observations raise the question if the immune effects may, at least in part, involve the accumulation of dCTL (Maciver et al., 2008). For example, studies *in vitro* show that strong T-cell stimulation – similar to that which might be elicited by CMV reactivation – enhances cellular glucose uptake, which can lead to the accumulation of readily activated memory T-cells that acquire resistance to cell death (Jacobs et al., 2008; Zhao et al., 2007). This presents a potential mechanism whereby hyperglycemia may amplify the CMV-induced accumulation of dCTL.

Therefore, the aim of the current study was to examine the relationship between glucose metabolism (i.e., HbA1c, fasting glucose, and diabetic status) and EM and EMRA T-cell numbers in a large sample of CMV+ and CMV– individuals. It was hypothesized that the effects of CMV infection on dCTL numbers would be enhanced in CMV+ individuals that show evidence of elevated glucose. Additionally, other factors associated with hyperglycemia, including markers of dyslipidemia (i.e., elevated circulating triglycerides and LDL

cholesterol, and lower HDL cholesterol) and elements of the metabolic syndrome (Ahmad Khan, 2007; Vinodmahato et al., 2011), may also contribute to increased dCTL accumulation, and were determined as well.

Materials and Methods

Participants

The present study was conducted among employees (N=1103; 88% male; mean age 40; range 18-64) of a large European airplane manufacturer in the south of Germany who took part in a voluntary company health check in 2011. Participant characteristics are presented in Table 6.1. Participants received a personalized comprehensive health report. All data was anonymized before analyses, and this study was approved by the ethical committee of the Medical Faculty Mannheim, Heidelberg University. All participants signed informed consent.

Procedures

Participants arrived at a location away from their usual workplace in the morning between 06:45h and 08:45h for their health check. After a fasting venous blood draw and medical examinations, participants were seated in a quiet room to fill out questionnaires measuring demographic, medical, and health behavior data. Anthropometric (e.g., height, weight, waist and hip circumference) and blood pressure measurements were carried out by trained study personnel. Demographic data, including age, gender, and marital status, along with SES indicators (measured as hierarchical job position, manual occupation, and shift work), self- and doctor-diagnosed medical conditions, and lifestyle factors (e.g., smoking, alcohol intake, exercise) were obtained by questionnaires used and validated in the MONICA study (Jönsson et al., 1999).

Flow cytometry

Flow cytometry was carried out as described in Chapter 5 (see pg. 74-75).

Cytomegalovirus (CMV) status determination

Fasting plasma samples were stored in small aliquots at -80°C until analysis. Evidence of previous CMV infection (serostatus) was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (BioCheck, Inc., CA, USA) according to manufacturer instructions. Optical density values obtained from participant samples were fitted to a standard curve. These concentrations were then compared to a cut-off value to compute CMV index scores. Participants with a borderline seropositive result, representing a calculated index score >0.85 and <1.15 , were retested (N=9). If they remained borderline, subjects with index scores above and below 1.00 were considered positive (CMV+) and negative (CMV-) respectively, as per manufacturer instructions. The sensitivity, specificity, and accuracy of the test are reported as 95.0, 96.7 and 96.0%, respectively.

Biochemical analyses

HbA1c, fasting glucose, triglycerides, low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) and high-sensitivity C-reactive protein (hs-CRP) were measured by an accredited clinical laboratory (Synlab Laboratories, Augsburg, Germany; <http://augsburg.synlab.de>) according to standard laboratory procedures complying with International Organization for Standardization norms (DIN EN ISO 15189). HbA1c was measured by a second-generation hemoglobin A1c immunoassay (Roche Diagnostics, Mannheim, Germany) and fasting glucose was measured by the glucose hexokinase enzymatic assay (Olympus Glucose OSR6121), in accordance with the latest standardized guidelines and

recommendations for laboratory analysis in the diagnosis of diabetes (Sacks et al., 2002).

Cholesterol and triglycerides were automatically measured enzymatically (Cobas 8000 analyzer, Roche Diagnostics, Mannheim, Germany). HDL-C was measured using a competitive homogeneous assay (Roche Diagnostics, Mannheim, Germany), and LDL-C was calculated using the Friedewald equation (Friedewald et al., 1972). These values were also used to calculate the ratio of LDL-C to HDL-C.

Diabetes & metabolic syndrome classification

Diabetes was classified in accordance with to the American Diabetes Association guidelines as individuals with fasting glucose ≥ 126 mg/dL and/or HbA1c $\geq 6.5\%$ in the absence of known diabetes. Those with self-reported doctor-diagnosed diabetes were also classified as diabetic. Pre-diabetes was classified as fasting glucose between 100 and 125 mg/dL and/or HbA1c between 5.7% and 6.4% (American Diabetes Association [ADA] 2013). The remaining normal-glycemic individuals, therefore, had fasting glucose and HbA1c levels < 100 mg/dL and $< 5.7\%$, respectively. The metabolic syndrome components were assessed as the following: (1) waist circumference > 102 cm (men) > 88 (women); (2) plasma triglycerides > 150 mg/dL; (3) plasma HDL-C < 40 mg/dL (men) < 50 (women); (4) blood pressure ≥ 130 (systolic) and/or ≥ 85 (diastolic) mmHg; (5) plasma fasted glucose ≥ 100 mg/dL. Each of these components were dichotomized (yes or no), and were added together to create a metabolic syndrome component score (range = 0-5). Those with a score ≥ 3 were classified as having metabolic syndrome (Grundy et al., 2005).

Statistical Analysis

To approximate a normal distribution of the variables used in the current analyses, we applied transformations based on information criteria obtained from the Ladder-of-Powers in STATA version 12 (StataCorp LP, College Station, TX). The transformation with the least statistical deviation from a normal distribution, indicated by the smallest χ^2 (or the most non-significant p-value) was used, as recommended (Gould & Hilbe, 1991). Missing data (<7% for all variables) was handled by multiple imputation in SPSS version 20 (IBM-SPSS, Chicago, IL, USA). Briefly, a fully conditional specification method was automatically chosen to replacing missing data. In this method, each variable was fit in a univariate (single dependent variable) model using all other available variables in the model as predictors, and missing values were imputed for each variable being fit. Linear and logistic regressions were used for continuous and categorical variables, respectively. Relevant variables with already complete data were entered only as predictors to improve estimates. After 10 iterations for each of 5 imputation datasets, the pooled estimates were used for all subsequent analyses below.

Firstly, participant characteristics, in terms of demographics and lifestyle behaviors, were compared between CMV+ and CMV- individuals. T-tests and χ^2 analyses were used for continuous and categorical variables respectively.

Secondly, differences in CMV status with HbA1c, fasting glucose and diabetic status were explored using binary logistic regressions. CMV status was entered as the dependent variable, and each of the above factors were entered, in turn, as an independent variable. Potential confounders known to impact CMV infection and reactivation were statistically controlled, including age, gender, marital status, socioeconomic status (job status, manual occupation) (Savva et al., 2013; Simanek et al., 2011), and lifestyle factors (smoking, alcohol

intake, BMI, and physical activity) (Hausenloy & Yellon, 2008) in stepwise models. Model 1 was adjusted for age and gender. Model 2 was additionally adjusted for marital status and SES (job status and manual occupation). Model 3 was further adjusted for smoking, alcohol, BMI and physical activity. These models were used throughout the remaining analyses.

Thirdly, numbers of CD8⁺ EM and EMRA T-cells were compared between levels of glycemic control, indicated by diabetic classification, using analysis of (co)variance (AN(C)OVA). These analyses were stratified by CMV status and the abovementioned potential confounders were entered as covariates (Models 1-3).

Finally, linear regressions were used to explore the associations between HbA1c, fasting glucose, and EM and EMRA T-cell subset numbers; potential confounders were entered as covariates using the same models as above.

The above analyses were repeated with each of the dyslipidemia and CVD risk factors (i.e., total cholesterol, LDL-C, HDL-C, the ratio of LDL-C to HDL-C and triglycerides) entered separately as independent variables. HbA1c was added as an additional adjustment to significant associations to clarify the impact of glucose levels on lipid metabolism. All analyses were performed with SPSS version 20 (IBM-SPSS, Chicago, IL, USA).

Results

Participant characteristics

As shown in Table 6.1, 400 (36%) of the participants were CMV+. On average, CMV+ participants tended to be older and female. They were also more likely to be current or former smokers, to drink less frequently, to have lower socioeconomic status (low job status and more manual occupations and shift work). There was no difference in the amount of cigarettes smoked (among smokers), BMI, waist-to-hip ratio, or physical activity (all p 's > .10;

Table 6.1). Tabulation of metabolic risk factors revealed that N=290 (26.3%) individuals met the criteria for metabolic syndrome classification. Diabetes classification resulted in three groups: 663 normal, 404 pre-diabetic, and 36 diabetic individuals. Because of the small number of diabetic individuals these were merged with the pre-diabetic group, and labeled “hyperglycemic”.

Glycemic control, CMV infection and dCTL numbers

Unadjusted analyses showed that CMV+ individuals were more likely to have higher levels of HbA1c and to be classified as hyperglycemic (i.e., pre-diabetic or diabetic) (Table 6.2). In binary logistic regressions, these associations of HbA1c and hyperglycemic status with CMV infection status were reduced to non-significance after adjustment for age and gender (Model 1) and socio-demographic factors (Model 2), respectively. Figure 6.1 shows the unadjusted comparisons of EM and EMRA T-cell numbers stratified by glycemic status and CMV infection. In all subjects, individuals classified as hyperglycemic had 26.6% higher numbers of EM (110.2 versus 87.0 cells/ μ L) and 41.2% (218.1 versus 154.5 cells/ μ L) higher EMRA T-cells (both $p<.001$), than normoglycemic participants.

When further stratified by CMV status, the results showed that EMRA T-cells were significantly higher in hyperglycemic versus normoglycemic CMV+ individuals ($p<.001$), while no such difference was observed in the CMV– group (Figure 6.1). This effect survived full adjustment, and was accompanied by a significant CMV status by glycemic status interaction for EMRA levels ($F(1,982)=4.65$, $p=.031$). These effects also did not appear driven by the small group of diabetic individuals, as identical results were found when diabetics were excluded from analyses (data not shown). Analyses adjusted for age and gender showed that

EM T-cells were not significantly higher in hyperglycemic individuals, compared to the normoglycemic group.

Linear associations with dCTL numbers

Table 6.3 shows the linear associations of metabolic factors with dCTL numbers. Overall, higher levels of continuous HbA1c were associated with increased numbers of dCTL after adjustment for age, gender, marital status, and SES (job status and manual occupation) (Model 2). These associations also remained significant after additional adjustment for lifestyle factors (Model 3). Fasting glucose was not significantly associated with dCTL numbers in all subjects (Table 6.3). When analyses were stratified by CMV status, the relationship between HbA1c and increased dCTL numbers remained in CMV+, but not CMV– individuals.

Dyslipidemia factors

Unadjusted comparisons of dyslipidemia factors between CMV+ and CMV– individuals revealed that CMV+ individuals also had a higher likelihood to have HDL-C levels that fell within the metabolic syndrome classification range (men: <40 mg/dL; women: <50). However, none of the other metabolic characteristics, including metabolic syndrome classification, differed by CMV status in unadjusted analyses (Table 6.2). After progressive adjustment for possible confounders (Models 1-3), the above association with the metabolic syndrome HDL-C category was no longer significant, but increased levels of continuous HDL-C became significantly associated with CMV infection (OR (95% CI)=0.55 (.319-.960), $p=.035$); that is, higher HDL-C (natural log) predicted a lower risk of CMV infection.

In all subjects, linear regressions showed that higher levels of triglycerides were associated with increased numbers of dCTL, after adjustment for possible confounders (Models 1-3) (Table 6.3). Lower HDL-C and a higher LDL-C/HDL-C ratio were also significantly associated with increased EM and EMRA numbers after adjustment for socio-demographic factors (Model 2). Except for the LDL-C/HDL-C association with EM T-cells, these relationships were attenuated by additional adjustment for lifestyle factors (Model 3). Total cholesterol and LDL-C were not significantly associated with dCTL numbers in all subjects (Table 6.3).

When analyses were stratified by CMV status, the relationship between triglycerides and increased dCTL numbers was found only among CMV+ individuals. Additionally, in the CMV+ group, total cholesterol and LDL-C/HDL-C ratio were positively associated EM T-cell numbers after adjustment for socio-demographic factors (Model 2) (both $p < .05$), although only cholesterol remained significant after full adjustment (Model 3) (Table 6.3).

To test the influence of glucose metabolism on the significant lipid profile associations in CMV+ individuals (i.e., triglyceride and cholesterol), HbA1c was additionally entered into each of these fully adjusted models. After the addition of HbA1c to the model, the above associations between triglycerides and dCTL were attenuated, while the relationship between cholesterol and EM T-cells remained significant (data not shown). Among CMV- individuals, no significant associations were observed between any metabolic factors and dCTL numbers (Table 6.3).

Table 6.1: Participant socio-demographic and lifestyle characteristics.

	Total N=1103	CMV Status		p
		Positive N=400(36%)	Negative N=703(64%)	
Age	40.1 ± 11.0	41.5 ± 11.1	39.3 ± 10.8	<.001
Gender (% male)	87.7	84.5	89.5	.020
Married/Co-habiting (%)	77.0	80.4	75.0	.065
Job status (%)	--	--	--	.007
<i>Division/dept mgr</i>	4.8	3.5	5.6	--
<i>Project leader/process mgr</i>	15.3	13.9	16.2	--
<i>Worker (managerial)</i>	6.7	6.0	7.1	--
<i>Skilled worker (non-mgr)</i>	63.8	62.6	64.4	--
<i>Semi-skilled worker</i>	9.4	14.1	6.7	--
Shift worker (% yes)	28.1	33.8	24.9	.003
Manual occupation (% yes)	49.6	55.0	46.5	.008
Smoking (%)	--	--	--	.050
<i>Never smoker</i>	46.1	41.1	48.9	--
<i>Former smoker</i>	24.9	27.0	23.8	--
<i>Smoker</i>	29.0	31.9	27.3	--
Cigarettes per day (in smokers)	14 ± 8	15 ± 7	13 ± 8	.090
Alcohol (%)	--	--	--	<.001
<i>0-2x/month</i>	24.9	30.4	21.7	--
<i>1-2x/wk</i>	29.9	32.5	28.5	--
<i>3-7x/wk</i>	45.2	37.2	49.8	--
Body mass index (kg/m ²)	24.5 ± 4.0	24.5 ± 4.0	24.5 ± 4.1	.929
Waist-to-hip ratio	0.90 ± .08	0.90 ± .08	0.90 ± .07	.190
Leisure physical activity (h/wk)	7.0 ± 7.5	7.1 ± 9.4	7.0 ± 6.2	.307

All are unadjusted comparisons of participant characteristics. A *t*-test was performed on continuous variables and a χ^2 for categorical variables. Values are mean ± SD unless otherwise stated.

Table 6.2: Participant metabolic characteristics.

	Total N=1103	CMV Status		p
		Positive N=400(36%)	Negative N=703(64%)	
HbA1c (%)	5.61 ± .01	5.64 ± .02	5.60 ± .01	.047
HbA1c (mmol/mol)	37.84 ± .11	38.13 ± .18	37.67 ± .13	.060
Fasting glucose (mg/dL)	86.75 ± .29	87.28 ± .50	86.45 ± .36	.168
Total cholesterol (mg/dL)	202.80 ± 1.20	203.73 ± 2.00	202.27 ± 1.50	.591
LDL cholesterol (mg/dL)	123.58 ± 1.05	124.47 ± 1.76	123.08 ± 1.30	.580
HDL cholesterol (mg/dL)	54.06 ± .45	53.23 ± .78	54.53 ± .55	.068
LDL-C/HDL-C ratio	2.47 ± .03	2.54 ± .05	2.42 ± .04	.140
Triglycerides (mg/dL)	125.70 ± 2.41	130.13 ± 4.07	123.19 ± 2.98	.145
Diabetes classification (%)	--	--	--	.005
<i>Normal</i>	60.1	53.9	63.6	--
<i>Pre-diabetes</i>	36.6	41.9	33.7	--
<i>Diabetes</i>	3.3	4.3	2.7	--
Metabolic syndrome (% yes)	26.3	28.0	25.3	.368
<i>Waist circumference (% yes)</i>	52.1	54.0	51.0	.387
<i>Triglycerides (% yes)</i>	31.8	35.0	30.0	.101
<i>HDL-C (% yes)</i>	17.7	21.0	15.8	.036
<i>Blood pressure (% yes)</i>	52.5	49.5	54.2	.150
<i>Fasting glucose (% yes)</i>	10.0	11.0	9.4	.442

All are unadjusted comparisons of participant metabolic characteristics. A *t*-test was performed on continuous variables and a χ^2 for categorical variables. Values are mean ± SD unless otherwise stated.

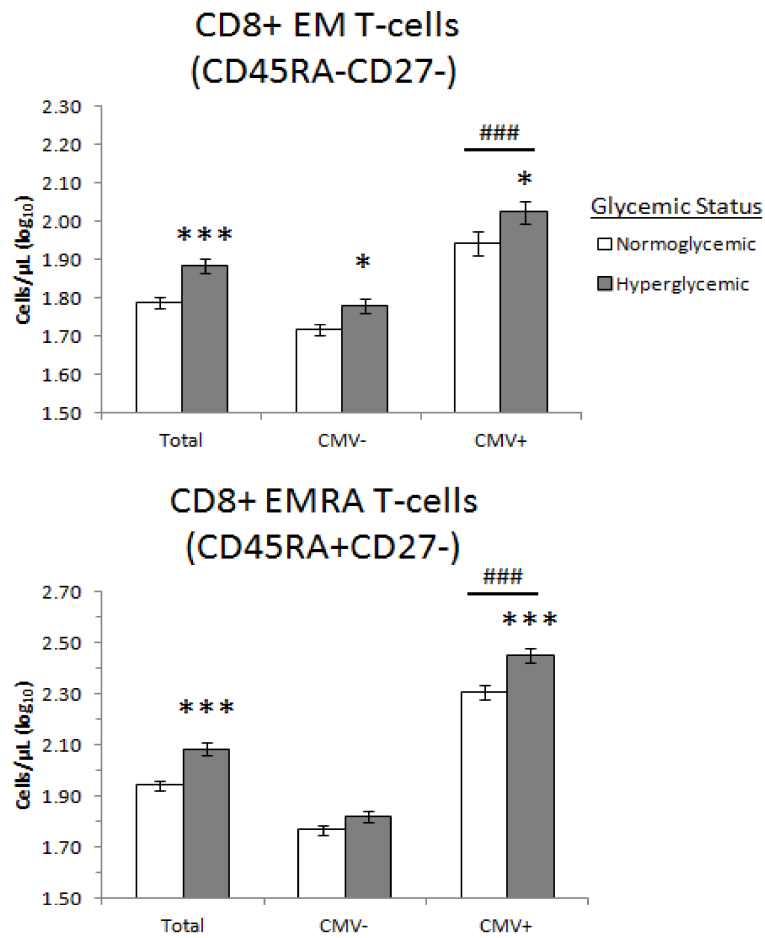


Figure 6.1: Unadjusted comparison of EM and EMRA T-cell subset numbers by glycemic status and CMV infection. Significantly different from normoglycemic at the * $p < .05$ & *** $p < .001$ level. Significantly different from CMV- at the ### $p < .001$ level. Values are mean \pm SEM.

Table 6.3: Unstandardized coefficients from linear regressions of metabolic factors and EM and EMRA T-cell numbers.

	All Subjects (N=1103)					
	EM (CD27-CD45RA-)			EMRA (CD27-CD45RA+)		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
HbA1c	2.03(.58)***	1.87(.59)***	1.54(.59)*	2.20(.77)**	2.05(.77)**	1.68(.78)*
Fasting glucose	2.15(2.06)	2.31(2.06)	3.23(2.07)	-2.92(2.69)	-2.74(2.70)	-1.72(2.73)
Cholesterol	.011(.01)	.013(.01)	.014(.01)	.012(.01)	.014(.01)	.015(.01)
LDL-C	.011(.01)	.013(.01)	.012(.01)	.011(.01)	.013(.01)	.010(.01)
HDL-C	-.134(.05)**	-.125(.05)**	-.062(.05)	-.155(.06)**	-.146(.06)*	-.076(.06)
LDL-C/HDL-C	.198(.07)**	.200(.07)**	.140(.07)*	.206(.09)*	.209(.09)*	.131(.10)
Triglycerides	.143(.05)**	.134(.05)**	.111(.05)*	.210(.07)**	.203(.07)**	.177(.07)*
	CMV Negative (N=703)					
	EM (CD27-CD45RA-)			EMRA (CD27-CD45RA+)		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
HbA1c	.974(.65)	.860(.65)	.458(.66)	.869(.23)	.939(.82)	.480(.84)
Fasting glucose	2.34(2.18)	2.40(2.18)	3.75(2.23)	-1.25(2.77)	-1.29(2.77)	.359(2.84)
Cholesterol	.001(.01)	.002(.01)	.001(.01)	.012(.01)	.011(.01)	.007(.01)
LDL-C	.004(.01)	.005(.01)	.003(.01)	.010(.01)	.009(.01)	.005(.01)
HDL-C	-.086(.05)	-.084(.05)	-.030(.05)	-.022(.06)	-.030(.06)	.024(.07)
LDL-C/HDL-C	.112(.07)	.117(.07)	.055(.08)	.083(.09)	.085(.09)	.012(.10)
Triglycerides	.066(.06)	.059(.06)	.028(.06)	.120(.07)	.129(.07)	.085(.07)
	CMV Positive (N=400)					
	EM (CD27-CD45RA-)			EMRA (CD27-CD45RA+)		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
HbA1c	3.11(1.03)**	3.02(1.03)**	2.75(1.03)**	3.02(1.00)**	2.92(1.01)**	2.90(1.02)**
Fasting glucose	2.41(3.82)	2.19(3.83)	2.36(3.83)	-3.86(3.72)	-3.78(3.74)	-3.40(3.77)
Cholesterol	.035(.02)*	.036(.02)*	.038(.02)*	.027(.02)	.029(.02)	.028(.02)
LDL-C	.026(.01)	.027(.01)	.027(.01)*	.018(.01)	.020(.01)	.019(.01)
HDL-C	-.112(.08)	-.103(.08)	-.044(.09)	-.109(.08)	-.102(.08)	-.064(.09)
LDL-C/HDL-C	.265(.12)*	.256(.12)*	.219(.13)	.211(.12)	.215(.12)	.175(.13)
Triglycerides	.224(.09)*	.218(.09)*	.201(.10)*	.228(.09)*	.222(.09)*	.206(.10)*

Model 1: Adjusted for age and gender

Model 2: Model 1 with additional adjustment for marital status and SES (job status and manual occupation)

Model 3: Model 2 further adjusted for smoking, alcohol, BMI and physical activity.

Values are beta (standard error). Significant at *p<.05, **p<.01, & ***p=.001.

Discussion

A hallmark of an aging immune system is the accumulation of differentiated CD8+ T-cells, which is strongly enhanced in CMV-infected individuals. The present study

demonstrated that impaired glycemic control, measured as HbA1c levels, is associated with elevated numbers of dCTL in CMV+ individuals, but not in those who are CMV-. This association was robust to adjustment for demographics, SES, and lifestyle factors. Thus, the present findings are the first to provide evidence that glycemic control may contribute to immunosenescence by amplifying the effects of CMV on T-cell differentiation. This mechanism may contribute to the impaired immunity seen in hyperglycemia and diabetic patients, and may possibly be a pathway linking CMV to increased CVD risk.

HbA1c may be positively associated with dCTL in CMV+ individuals due to a synergistic impact on both the extent of the CMV replication and the T-cell response to CMV activity. Elevated glucose levels may (1) directly and indirectly increase the efficacy and frequency of CMV replication and (2) allow for enhanced T-cell responses to CMV. Firstly, CMV-induced upregulation of the glucose transporter, GLUT4, enhances glucose uptake (Landini, 1984; Mcardle et al., 2012; Yu, Maguire, et al., 2011). This excess glucose influx is diverted towards the biosynthesis of fatty acids, which are used to directly increase viral production and enhance infectivity (Chambers et al., 2010; Munger et al., 2008; Seo & Cresswell, 2013; Spencer et al., 2011; Yu, Clippinger, et al., 2011). Indirectly, elevated glucose promotes the production of reactive oxygen species (ROS), which is known to facilitate the reactivation of CMV by stimulating the CMV promoter region (a required first step for CMV reactivation) (Cohen et al., 2011; Demissie et al., 2006; Jaganjac et al., 2010; Jain, 1989; Muriach et al., 2008; Self-Medlin et al., 2009). Secondly, evidence from *in vitro* studies demonstrate that strong, repeated antigen receptor stimulation (e.g., by CMV) can lead to the upregulation of GLUT1, and enhanced glucose uptake by T-cells. Excess glucose uptake was shown to parallel increased T-cell activation, pro-inflammatory cytokine

production, as well as an elevated threshold for cell death (Jacobs et al., 2008; Maciver et al., 2008; Ramakrishnan et al., 2010; Zhao et al., 2007). Although not directly investigated in the present study, these represent biologically plausible mechanisms through which elevated glucose could augment dCTL numbers via CMV activity.

The attenuation of the relationship between hyperglycemia and CMV infection by socio-demographic factors is in line with other studies finding null associations after similar adjustments (Haeseker et al., 2013; Hausenloy & Yellon, 2008; Lutsey et al., 2009), but disagrees with somewhat divergent epidemiological data by Chen et al. (2012) from very old (85 years) adults. This finding suggests that unadjusted differences in glucose by CMV status are likely a consequence of common predisposing factors, rather than being causally linked. Indeed, both CMV infection and dysregulated glucose metabolism are more common in those with a variety of pre-existing health risk factors. For example, CMV has a non-random distribution in the population, and infection is particularly prevalent among those who are typically older, current smokers, have low SES (job status, education) or ethnic minority status (Dowd et al., 2009; Simanek et al., 2009; Stadler et al., 2010). On the other hand, the robust linear relationships between HbA1c and EM and EMRA T-cells found with only within CMV+ individuals suggest glycemic status may contribute to immune responses to CMV reactivation rather than initial infection.

The current positive relationship between markers of dyslipidemia and numbers of dCTL among CMV+ individuals appears to partly be a by-product of the intrinsic link between HbA1c and lipid metabolism found in both diabetic (Ahmad Khan, 2007; Khan et al., 2007; Ladeia et al., 2006; Vinodmahato et al., 2011) and non-diabetic adults (Selvin et al., 2010). This relationship is evidenced by the null associations between triglycerides and dCTL

numbers among CMV+ individuals after additional adjustment for HbA1c in the current study. In contrast, the cholesterol-EM association survived additional adjustment for HbA1c, making a more direct effect of CMV more plausible. The factors underlying this association are not clear, but could reflect the manifestation of a host defense mechanism to limit CMV infectivity. As intracellular cholesterol is necessary for enhanced CMV virus production and effective entry into other cells (Chukkapalli et al., 2012; Heaton & Randall, 2011; Williamson et al., 2011; Zhang et al., 2013), the reduction of cellular cholesterol uptake by the host cell could explain the association of elevated cholesterol with EM accumulation here (Gudleski-O'regan et al., 2012; Juckem et al., 2008). This does not, however, rule out the strong relationship between lipid and glucose metabolism, and combined effects are also possible.

The current finding that CMV+ individuals with higher HbA1c and CVD risk factors (cholesterol and triglyceride levels) had elevated dCTL provides further evidence to a common impact of both CMV (Gkrania-Klotsas et al., 2012; Savva et al., 2013; Van De Berg et al., 2012) and dysregulated glucose metabolism (Adams et al., 2009; Barr et al., 2009; Barr et al., 2007; Eskesen et al., 2013) on cardiac health. Consistent with this notion, studies have found associations between circulating dCTL, CMV-specific T-cell responses and increased CVD risk factors, such as heart valve calcification, carotid artery thickness, and increased blood pressure (Hsue et al., 2006; Terrazzini et al., 2013; Winchester et al., 2011). Taken together, these findings support the hypothesis proposed by Simanek et al. (2011) that CMV infection and inflammation partially impact mortality risk via their combined contribution to other CVD risk factors, and further propose dysregulated glucose metabolism and increased dCTL as additional mechanisms.

There are a number of limitations with the current study that should be acknowledged. Firstly, the low number of diabetic participants precluded their inclusion as a separate group; comparisons were instead performed between normoglycemic and a merged hyperglycemic group. Information about whether these participants had Type I or Type II diabetes was also not available. However, clinically relevant markers of immunosenescence (e.g., short telomere length) are observed even at early stages of glucose dysregulation (Adaikalakoteswari et al., 2007; Salpea & Humphries, 2010; Salpea et al., 2010), and the associations found in hyperglycemic individuals were not altered by the removal of the diabetic group from the analyses. Secondly, there was no direct measure of subsequent CMV reactivation. Nevertheless, the selective EMRA T-cell accumulation is almost exclusively associated with CMV infection, and there is a strong empirical basis to suggest that these associations are reflections of CMV activity (Cantisán et al., 2010; Chidrawar et al., 2009; Derhovanessian et al., 2011; Turner et al., 2014; Van De Berg et al., 2008; Van Lier et al., 2003). However, future studies should include more direct measures of CMV reactivation (e.g., quantitative CMV-specific antibody levels) for comparison. Finally, due to the cross-sectional nature of the study, we are unable to discern cause-and-effect relationships. Given the complex interplay between components of the immune system, metabolic factors, CMV, and aging, as well as the contribution of potential intermediaries such as oxidative stress and inflammation to these processes, bi-directional or cyclical relationships between these factors cannot be ruled out.

In conclusion, in the largest study to-date, we observed associations between measures of glucose metabolism (i.e., HbA1c and diabetic status), dyslipidemia (total cholesterol and triglycerides), and dCTL subsets among CMV+ individuals. These associations with HbA1c

withstood adjustment for demographic, SES, and lifestyle factors known to impact both CMV infection and glucose metabolism, demonstrating a robust association. Overall, it appears that these metabolic factors act reciprocally with CMV to amplify the accumulation of EM and EMRA CD8⁺ T-cells, and represent potentially biologically relevant pathways underlying the CMV-induced acceleration of immunosenescence.

CHAPTER 7 : GENERAL DISCUSSION

Summary of main findings

This thesis set out to explore the psychosocial and behavioral determinants of immune aging in a large sample of working-aged adults. Specifically, CMV infection and its reactivation were considered as a shared pathway between these factors and immunosenescence. The data presented in the first two empirical chapters (Chapters 3 and 4) were drawn from MICS 2007 using as outcomes, CMV serostatus and CMV reactivation, measured as levels of CMV-IgG antibody. Chapter 3 shows that increases in self-reported measures of psychological stress are associated with higher CMV-IgG levels among those infected. On the other hand, it was the socio-demographic and lifestyle factors, (i.e., SES, marital status, and smoking) that associated with higher odds of initial CMV infection. These associations appeared to be independent of HPA-axis and inflammatory activity. Chapter 4 revealed that those reporting higher levels of conscientiousness had lower CMV-IgG levels, while those higher in neuroticism had increased odds of CMV infection. These associations were apparent after mutual adjustment for the other Big 5 personality traits and were not explained by SES or lifestyle factors.

The data presented in chapters 5 and 6 were drawn from the MICS 2011 dataset using the accumulation of highly-differentiated T-cells as a measure of immunosenescence. Chapter 5 revealed that increased frequency of physical activity is associated with decreased accumulation of highly-differentiated T-cells, which was observed only in those in the upper two age tertiles. Adjustment for CMV status attenuated all linear associations with highly-differentiated T-cells, except the lower EMRA $\gamma\delta$ T-cells observed with increased moderate physical activity. Chapter 6 showed that impaired glycemic control interacted with CMV to

enhance the accumulation of highly-differentiated T-cells among CMV+ individuals. The above associations remained significant after adjustment for potential confounders, suggesting a robust effect.

Collectively, the data in this thesis provide evidence in support of the notion that whatever dictates CMV reactivation may, in turn, influence the aging of the immune system (Nikolich-Zugich, 2008). Specifically, it appears that psychosocial and behavioral factors may be such relevant determinants of CMV activity, and thereby, of immunosenescence. In recent years it has become increasingly clear that incessant antigenic stimulation by herpes viruses, in particular CMV, is a major driving force of senescence within the T-cell compartment. This immune dominance is evident among those infected in the dramatic expansion of CMV-specific T-cells displaying a highly-differentiated phenotype (e.g., CD45RA+/-CD27-); a hallmark of immune aging. Thus, the associations found between these psychosocial and behavioral factors and these outcomes may help to substantiate this claim.

Psychosocial and behavioral factors interact to determine CMV activity

The data appear to reveal a pattern, whereby some of these factors promote CMV reactivation (psychological stress and dysregulated metabolism), while others play a protective role (conscientiousness and physical activity), working to keep the virus in latency. Considering the potentially opposing effects of the factors studied here, it is tempting to speculate that CMV reactivation may reflect the imbalance of this dynamic relationship. Below, I describe how the factors increasing or decreasing CMV reactivation are interconnected, and speculate that these factors may work against one another to co-determine the set-point for CMV activity and the progression of immunosenescence.

Psychological stress and glucose metabolism

Observations from the stress-metabolism literature suggest that the observed increase in CMV activity with both stress and HbA1c may be two sides of the same coin; that is, chronic psychological stress could possibly exacerbate the effects of dysregulated glucose metabolism. Psychological stress is characterized by a shift in the hormonal balance towards an increasingly catabolic state (Epel, 2009), resulting in excessive release of endogenous substrates, including glucose, into the periphery, disproportionate to metabolic needs (Balanos et al., 2010; Carroll et al., 2009; Laugero, 2008). Indeed, a recent study by Li et al. (2013) showed that increased work stress was associated with increased odds of pre-diabetes and diabetes in men. HbA1c reflects this chronic elevation in glucose and, consequently, has been proposed as a physiological marker of psychological stress (Schuck, 1998). Therefore, psychological stress may not only stimulate the CMV IE promoter region by mediators of the HPA- and SAM-axis (i.e., glucocorticoids and catecholamines), but also enhance CMV reactivation via stress-induced hyperglycemia and the related oxidative stress pathways described in chapter 6.

Conscientiousness and physical activity

The protective effects of conscientiousness and physical activity also have complimentary features, and together, may oppose the above effects of stress and dysregulated metabolism on CMV reactivation and immunosenescence. Certainly, conscientiousness is associated with more favorable metabolic profile, and may exact its action, in part, through health-behaviors, including better dietary choices (Tiainen et al., 2013; Wen et al., 2015), abstinence from smoking (Martin et al., 2007) and importantly, a physically active lifestyle (Chatzisarantis & Hagger, 2008; Rhodes & Smith, 2006). Physical exercise is also known to

counteract the debilitating physiological effects of psychological stress on the body (Phillips et al., 2007). For example, chronic stress has been associated with increased adiposity, oxidative stress, circulating levels of cortisol and pro-inflammatory cytokine production (Djuric et al., 2008; Epel, 2009; Laugero, 2008). Physical activity, in contrast, increases lipid metabolism, improves anti-oxidant capacity, increases DHEA production, and induces an anti-inflammatory environment with each successive bout (Lawler & Powers, 1998; Pedersen, 2009; Pedersen & Febbraio, 2008; Phillips et al., 2007; Rowinski et al., 2013). These consequences of chronic stress are known to increase CMV reactivation, and may therefore be a plausible mechanism by which the current results with conscientiousness and physical activity can be attributed.

Both conscientiousness and physical activity also effectively enhance stress resilience. Those high in conscientiousness are reported to have a reduced frequency of stress exposure, which may stem from decreased appraisal of stressors as threats, and to utilize better coping strategies (Bartley & Roesch, 2011; Gartland et al., 2012; Murphy et al., 2013). Physical exercise not only alleviates measures associated with chronic stress, such as anxiety and depression (Rethorst et al., 2009; Wipfli et al., 2008), but can also buffer physiological responses to acute psychological stressors. Although the literature regarding the immune system is largely mixed, evidence accrued from studies on the so-called ‘cross-stressor adaptation hypothesis’ supports the beneficial effects of an exercise training program on cardiovascular and endocrine stress responses to a subsequent acute social stressor (Klaperski et al., 2013, 2014). This protection against stress effects, including both chronic metabolic alterations and acute stress reactivity may reduce the toll that psychological stressors take on

the body and could, in part, account for the lowered CMV reactivation observed with increased conscientiousness and physical activity.

On the other hand, evidence exists that supports a bi-directional relationship between these factors. Chronic stress has been shown to affect the frequency of participation in physical activity and exercise (Martins & Lopes, 2013; Stults-Kolehmainen & Sinha, 2014). For instance, a recent survey across 16 US states revealed that those individuals with serious psychological distress were less likely to meet recommended levels of physical activity (Okoro et al., 2014). Additionally, increased depressive symptoms have been found to mitigate the anti-inflammatory benefits of leisure-time physical activity on hs-CRP levels (Suarez et al., 2013). It is also worthy of mention that among people high in conscientiousness (i.e., one standard deviation above the mean), chronic interpersonal stress was associated with increased glucocorticoid resistance (Murphy et al., 2013). In these situations, the effects of stress would tip the scales in favor of immune dysregulation and could culminate in increased CMV reactivation. These findings reinforce the idea that these factors are in competition with one another to establish equilibrium; the outcome of which is reflected in CMV reactivation.

Determinants of CMV infection

CMV infection may represent a biological link between psychosocial and behavioral factors, disease and mortality. Across both the 2007 and 2011 cohorts, those infected with CMV are characterized as older, married individuals that have lower socioeconomic status and make poorer lifestyle choices (e.g., smoking). In the 2007 sample, CMV+ individuals were more neurotic than those uninfected, and in the 2011 sample, they were more likely to be female, less physically active, to drink alcohol less frequently, to have lower HDL-C levels and to be classified as hyperglycemic or diabetic. Overall, this archetype of CMV-infected

individuals bears striking resemblance to the profiles of high-risk populations for several comorbidities, including cardiovascular disease, cognitive decline, cancer, as well as increased mortality (Hausenloy & Yellon, 2008). That is, the factors predicting these disease states likewise predispose individuals to initial CMV infection. While these factors may progressively lead to disease by themselves, additional CMV infection would drastically enhance pathogenesis via accelerated immunosenescence, providing a ‘double-whammy’. This observation could arguably explain links between these predictors of CMV infection and increased mortality risk. For instance, the health effects of neuroticism, agreeableness and low SES have been found to be largely explained by health-behaviors (i.e., smoking, and physical inactivity), which are, in turn, associated with increased CMV infection odds (Chapman et al., 2010; Mroczek et al., 2009; Shipley et al., 2007). Although not studied explicitly here, the considerable overlap between these factors gives credence to the notion that CMV infection may co-determine the pathogenesis of disease and increased mortality.

These findings suggest that CMV serostatus could also be used as a sensitive biomarker for disease risk. Interestingly, CMV infection was accompanied by increases in some risk factors for cardiovascular disease, like low HDL-C, diabetes, low SES, smoking, and less alcohol intake, but did not differ in other classic risk factors, such as obesity (i.e., BMI, waist-to-hip ratio), triglycerides, total cholesterol, blood pressure, resting heart rate. If applied as a screening tool, CMV serostatus may discriminate between at-risk populations in the absence of overt manifestations of physical dysregulation. This prospect could better inform treatment protocols, allow for earlier intervention and improve the prevention of numerous diseases. Therefore, it is proposed here that the public health implications of including CMV in basic screening panels in hospitals could be significant. Some potential

actions could be to supplement standard regimens with stress reduction, exercise, or nutritional interventions to reduce the reactivation of CMV in those infected, possibly improving prognosis for their other ailments. Monitoring CMV-IgG levels could potentially help assess the efficacy of such interventions on immune functioning (cf. Moro-Garcia et al., 2013).

Limitations and future directions

Limitations

A limitation of this thesis is that, due to the available data for each outcome (i.e., CMV-IgG and T-cell accumulation), these studies were not able to directly link all the psychosocial and behavioral factors to immunosenescence in one model. Although subcomponents of this model have been confirmed (i.e., stress and latent herpes reactivation, and the role of CMV infection in development of immunosenescence), the full model still awaits empirical scrutiny. The interconnectedness of the factors examined here provides a strong basis for their inclusion as predictors in future studies. These studies may benefit from a structural equation modelling approach with latent variables representing the constructs of psychological stress, personality, SES, physical activity, and metabolism to clarify their relationship to CMV infection, reactivation, and immunosenescence. This scrutiny seems worthwhile as confirmation will have significant implications for our understanding of the links between stress and immunity. One of its main implications would perhaps be that the host's infection history is an important, and thus far overlooked, mediator of these links.

Another limitation is that the cross-sectional study design does not allow for causality to be inferred. The hypothesized model is based on the current literature demonstrating CMV reactivation as a result of the factors examined, and the data presented here are consistent with

that orientation. Nevertheless, it still remains conceivable that the relationships between these factors and CMV are bi-directional or even cyclical. The latter has been demonstrated for another herpes virus (HSV-2), whereby reactivation was both a cause and a consequence of psychological distress (Strachan et al., 2011). Two major challenges for future investigations seeking these causal confirmations lie in the ubiquitous nature of CMV in the population (Dowd et al., 2009) and uncertainty about how long an individual has had CMV (due to early-life infection). While the former is difficult to tackle, attempts to address the latter have utilized longitudinal study designs in newly-infected transplant patients to circumvent these issues, but the populations presumably have many other potential confounders, preventing generalization to healthy adults. Perhaps future studies could measure CMV activity in parallel with the abovementioned factors (pre- and post-infection) among incoming university freshmen to discern the effects of infection and/or reactivation on the parameters studied herein and *vice versa*.

A potential next step

One consideration that could build upon the findings here is that CMV may also alter psychobiological responses to stress; in particular, stress-induced lymphocytosis. Lymphocytosis is probably one of the best documented effects of stress on the immune system; it involves a rapid (within minutes) increase in the absolute number of lymphocytes in the peripheral blood (Benschop et al., 1996; Bosch et al., 2005; Dhabhar & McEwen, 1999). Lymphocytosis is driven by β -adrenergic mechanisms, and the lymphocyte subsets that show the strongest mobilization during stress exhibit the highest β_2 -adrenergic receptor (β_2 AR) density and cytotoxic ability, such as natural killer (NK) cells and $CD8^+$ T lymphocytes (CTLs), and also $\gamma\delta$ T-cells and the small subset of cytotoxic $CD4^+$ T-cells (Anane et al.,

2009; Campbell et al., 2009; Elenkov et al., 2000). Coincidentally, these are the same cell types that become strongly enriched in peripheral blood as a result of CMV infection. Indeed, Riddell (2010) found in CMV-positive hosts that CMV-specific CTLs had a greater propensity to mobilize than total CTLs in response to acute stress, and that they contained a larger proportion of EM (CD45RA⁻CD27⁻) and EMRA (CD45RA⁺CD27⁻) cells (Riddell, 2010). This strong and robust mobilization of the stress sensitive, highly-differentiated EM and EMRA populations evoked by acute psychological stress is likely explained by their increased expression of the β_2 AR (Dimitrov et al., 2009; Dimitrov et al., 2010).

While a fascinating physiological phenomenon, it is still unknown if the enhanced stress-induced recruitment of cytotoxic cells in CMV+ individuals has any immunological or clinical impact, such as regulation of inflammation or viral control. There is evidence to suggest that the enhanced β -adrenergic sensitivity of CMV-specific CTLs may directly impact viral control, as adrenergic stimulation of T-cells can alter a variety of effector functions including proliferation, lytic activity, and cytokine production (Bartik et al., 1993; Borger et al., 1998; Glaser & Kiecolt-Glaser, 2005; Gratama et al., 2008; Hatfield et al., 1986; Kalinichenko et al., 1999; Leo & Bonneau, 2000; Lilleri et al., 2008; Morita-Hoshi et al., 2008; Ozdemir et al., 2002; Sarid et al., 2001, 2004; Sekut et al., 1995). One may speculate, therefore, that the amplification of cytotoxic lymphocyte mobilization during stressful events in CMV+ individuals may be conducive to elevated systemic inflammation whilst also decreasing antigen-specific immunity; both of which are hallmarks of immunosenescence. Future studies correlating the CMV-induced accumulation of highly-differentiated T-cells, the frequency and magnitude of responses to acute stressors, and outcomes in clinical populations,

such as cardiovascular disease, cancer and autoimmune disease patients could be informative in this regard.

Conclusion

The data in this thesis support psychosocial and behavioral factors as determinants of immune aging, with CMV infection and its reactivation representing a common biological pathway. Taken as a whole, the results show a pattern that can be likened to a reverberating circuit in which the factors studied here may induce, perpetuate or combat CMV reactivation and immunosenescence. First, inter-related predisposing factors, such as neuroticism, low socioeconomic status, smoking, and physical inactivity, increase the odds of initial CMV infection. Once infected, elevations in other factors, such as psychological stress and impaired glycemic control, occur concomitantly with CMV reactivation from latency, thereby accelerating the accumulation of highly-differentiated T-cells. The by-products of this process, such as inflammatory mediators, provide positive feedback to the cycle and propagate more damage, resulting in increased disease and mortality risk. Other factors, such as conscientiousness and physical activity, may work to interrupt this detrimental cycle and restore balance to the system. Thus, the current results suggest that CMV infection history may play an insidious role in determining disease and mortality. There may be hope in the form of improved screening, intervention and prevention of CMV effects on immune aging; this may be of particular relevance in immune-compromised individuals, such as HIV patients, transplant recipients and critically-ill populations. Future studies in these areas should take a more integrated, rather than piecemeal, approach while accounting for the impact of CMV activity on immunity, health and longevity.

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